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GROWTH AND YIELD RESPONSE OF SELECTED SPECIES OF AFRICAN LEAFY VEGETABLES INFESTED WITH ROOT KNOT NEMATODES (*Meloidogyne incognita*)

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Abstract

African indigenous leafy vegetables (AILVs) are an important commodity in the diet of many African communities. Most of the vegetables are grown by low-income small holder farmers and thus, play a crucial role in food security and in improving the nutritional status of poor families. However, root knot nematodes are a major hindrance to production with yield losses of 80 to 100 percent being recorded on some of the vegetables depending on susceptibility and inocula levels in the soil. The objective of this study was to investigate the effect of root knot nematodes on the growth and yield of popular AILVs. A greenhouse experiment was conducted twice, where AILVs namely spider plant (*Cleome gynandra*), amaranthus (*Amaranthus hybridus*), African night shade (*Solanum nigrum*), cowpea (*Vigna unguiculata*), jute mallow (*Corchorus olitorius*) and sun hemp (*Crotalaria juncea*) were tested. The seeds for each vegetable were planted in six pots out of which three of the pots were infested with 2000 second stage juveniles of root knot nematodes and data on plant height, fresh and dry shoot weight, galling index, egg mass index and the second stage juvenile count was recorded and analyzed. The experiment was terminated at 60 days after planting. Fresh shoot weight was significantly ($P \leq 0.05$) different among the different AILVs which were infected by the root knot nematode. The highest fresh shoot weight reduction was recorded in cowpeas (26.2%), African night shade (21.9%) and jute mallow (19.3%) lower in inoculated compared to the non- inoculated plants. The lowest fresh shoot weight reduction was recorded in spider plant (5.3%), sun hemp (5.2%) and Amaranths (6.7%) compared to the non-inoculated plants. On a scale of 1-10, where 1 = resistant and 10 = most susceptible, galling index was 1.7 in amaranths and 7.0 in the African night shade. Spider plant, sun hemp and amaranths had galling indices of 3, 2 and 1.7 respectively and were rated as resistant while jute mallow, cow pea and African night shade had galling indices of 6.7, 6.3 and 7 respectively and were rated as susceptible. The identified resistant varieties can be used as intercrops/rotation crops in agricultural production systems as a component of root knot nematode suppression in the soil.

Keywords: Root-knot nematodes, inoculum, resistance, Leafy vegetables.

1. Introduction

African indigenous leafy vegetables (AILVs) play an important role as food and nutritional security to many rural and urban households in Kenya (AVRDC, 2003). The crops also provide a source of income to resource-poor farmers with small land units since they are cheaper to produce compared to exotic crops such as maize (IPGRI, 2003). They are adapted to low-input agriculture, have a short maturity period and have a high potential for yield per unit area (Abukutsa-Onyango, 2004). In addition, the AILVs are richer in minerals such as calcium, iron and vitamins compared to exotic vegetables (Abukutsa-Onyango, 2003) and have been associated with several health benefits such as antioxidant, anticarcinogenic, analgesic and immunomodulatory properties (Kalpesh *et al*., 2008).

Insect pests, diseases and nematodes can cause upto 80% losses in vegetables yield (Cetintas and Yarba, 2010). Root knot nematodes (RKNs) mainly *Meloidogyne incognita*, *M.javanica* and *M.arenaria* are a major cause of yield decline in the production fields. They alter the plant physiology by producing specific enzymes that induce giant cell formation within the root at the feeding site (Karssen *et al.*, 2006). The giant cells then act as sinks by "attracting" energy rich plant metabolites, which are consumed by nematodes. The abnormal cells (Galls) disrupt moisture and nutrient transport within the plant thus interfering with their growth and photosynthesis (Anwar and McKenry, 2010). Root knot nematode (*Meloidogyne incognita)* is the most widespread and most injurious nematode to a wide range of crops in the tropics and subtropics (Atkins *et al*., 2004). Knowledge on the effects of root knot nematodes on the growth of African leafy vegetables is scanty. Research on AILVs, in Kenya, has concentrated on nutrient chemical composition neglecting biotic constraints in production. Identification of resistant indigenous leafy varieties would contribute greatly to the management of the pests in the cropping systems. Therefore, the objective of this study was to assess the effect of root knot nematodes on growth and yield of selected ALVs.

2. Materials and Methods

2.1 Preparation of root knot nematodes inocula

Extraction and preparation of nematode inoculum was carried out at Kabete plant pathology laboratories in the department of Plant science and crop protection. The eggs and second stage juveniles (J2) were obtained from the nematode infested spinach plants and soil at Kabete field station. Infected roots and soils were collected and used to prepare the inocula. The root maceration method described by Coyne *et al.* (2007) was used to extract nematode eggs and the juveniles.Briefly; roots were gently washed with tap water and cut into 1cm long pieces. About 20g of roots were weighed to which, a ratio of 1g of root to 20ml water and 0.5% sodium hypochlorite (NaOCl) was added to the root water mix. The mixture was loaded into a domestic blender and blended for 15 seconds at high speed (Hooper *et al.*, 2005) and the process repeated to obtain the required inoculum. The mixture was sieved and using a dissecting microscope the eggs and the second stage juveniles (J2) were counted to estimate the concentration per milliliter of the fluid from the sieving. The extracted juveniles were used to inoculate half of experimental plants grown in the greenhouse.

2.2 Experimental material

Screening of the selected species of African indigenous leafy vegetables (AILVs) was done in the greenhouse where artificial inoculation of nematodes was done in pots with growing plants. Six different Africa indigenous leafy vegetables were planted in pots containing sterilized soil which had a mixture of sand (Volcanic ash) and top forest soil mixed in the ratio of 1:3. The selected (AILVs) were sun hemp, jute mallow, amaranth, cow peas, spider plant and African nightshade each replicated thrice to determine their susceptibility or resistance to plant parasitic nematodes.

Seeds of the selected plant species were planted four per pot and watered on daily basis. After sprouting they were thinned to two seedlings per pot and CAN top dress fertilizer was added at a rate of 20 grams per pot. The nematode inoculum which was previously prepared was used to artificially inoculate the potted AILVs. The first inoculation with 2000 J2 juveniles was done ten days after planting on three pots per AILV species and the same was repeated two weeks later after the first inoculation (Bridge *et al.* 2007). The plants were monitored for symptoms such as changes in leaf colour, height, stem size and growth vigour. Soil and root samples were taken from the rhizosphere of the plants in each pot by gently removing the soil. Both roots and soil samples were placed in labeled polythene sample bags and transported to the laboratory in a cool box where the samples were stored at 10^oC before nematode bioassays were conducted. The roots were carefully and gently washed with tap water and they were blotted dry.

2.3 Parameters measured

The data on plant height on all AILVs in the pots was taken after every two weeks interval. The fresh and dry biomass weights on all AILVS in pots were also recorded 60 days after planting during flowering. The galling index rating was assessed using a chart illustrated by Coyne *et al.* (2007) with a scale ranging between 1 -10 where 1 indicated no galling and 10 indicated severe galling. After assessing the galling index the root knot nematodes juveniles (J2) were extracted by use of the modified Baermann technique (Hooper *et al*., 2005) to identify and count the nematodes. The egg mass index at a scale of 1-5 was also assessed. Galling index, egg mass index and the juveniles in the soil samples from each plant were used to rate nematode infestation and levels of infestation for the selected six African indigenous leafy vegetables to root knot nematodes and determine those which were susceptible or resistant to root knot nematodes.

2.4 Data analysis

Data on the counts was log transformed before being subjected to analysis of variance (ANOVA) using Genstat statistical software package (Lane and Payne, 2007). Significance of the differences between treatments was measured by *T*-test, while the treatment means were compared using Fisher's protected least significant difference (lsd) at p=0.05.

3. Results

Plants infected with the root knot nematodes recorded reduced plant heights compared to the untreated controls. The highest plant height reduction was recorded in African night shade (18.5 %) followed by cowpea(15.8%) then jute mallow (12.5%) and significantly (P≤0.05) different from untreated controls (Table 1).The lowest plant height reduction was recorded in sun hemp (2.9 %) followed by Amaranths(3.5%) then spider plant(4%) and were not significantly (P≤0.05) different from the untreated controls (Table1). Similar trend was observed when the experiment was repeated (Table 2). Plants infected with the root knot nematodes recorded reduced shoot weights compared to the untreated controls. Three plants recorded lowest fresh shoot reduction;Amaranthus (6.7%),spider plant (5.3 %) and sun hemp (5.2%) hence had no significant difference with the untreated controls while cowpea (26.4%), African night shade (21.9 %), jute mallow (19.3 %) had the highest shoot weight reduction showing significance (P≤0.05) differences (Table 3). A similar trend was observed when the experiments were repeated (Table 4).

Table 1: Effect of nematodes on the mean plant height and % reductions in height of the African indigenous leafy vegetables grown under greenhouse conditions in season one.

Parameter		Plant species					
		Amaranth (*Amaranth hybridus*)	Cowpea (*Vigna unguiculata*)	Sun hemp (*Crotalaria juncea*)	Jute Mallow (*Corchorus spp*)	Spider plant (*Cleome gynandra*)	African night shade (*Solanum nigrum*)
Plant height(cm)	Inoculated	43.1a	27.1a	46.7a	35.6 a	61.7a	41.4 a
	Non inoculated	44.7a	32.2b	48.1 a	40.7b	64.3a	50.8b
	% reduction	3.5	15.8	2.9	12.5	4	18.5
LSD(p≤0.05) value		1.8	4.64	1.58	3.61	2.83	6.42

Figures along a column followed by the same letter are not significantly different

Table 2: Effect of nematodes on the mean plant height and % reductions in height of the African indigenous leafy vegetables grown under greenhouse conditions in season two.

Parameter		Plant species					
		Amaranth (*Amaranth hybridus*)	Cowpea (*Vigna unguiculata*)	Sun hemp (*Crotalaria juncea*)	Jute Mallow (*Corchorus spp*)	Spider plant (*Cleome gynandra*)	African night shade (*Solanum nigrum*)
Plant height(cm)	Inoculated	38.8a	27.1a	44.8a	32.9 a	55.6a	41.5a
	Non inoculated	41.7a	36.3b	49.4a	42.6b	61.3a	60b
	% reduction	7.1	25.3	9.3	22.8	9.3	30.8
LSD(p≤0.05) value		3.2	7.41	4.72	6.12	5.76	8.41

Figures along a column followed by the same letter are not significantly different

Table 3: Effect of nematodes on the mean fresh shoot weights and % reductions in weight of the African indigenous leafy vegetables grown under greenhouse conditions in season one.

Parameter		Plant species					
		Amaranth (*Amaranth hybridus*)	Cowpea (*Vigna unguiculata*)	Sun hemp (*Crotalaria juncea*)	Jute Mallow (*Corchorus spp*)	Spider plant (*Cleome gynandra*)	African night shade (*Solanum nigrum*)
Fresh shoot weight(gm)	Inoculated	111.9 a	50 a	70.1a	65.4a	78.6a	83.4a
	Non inoculated	119.9a	67.8b	73.8a	81b	82.7a	105.3b
	% reduction	6.7	26.2	5.2	19.3	5.3	21.9
LSD(p≤0.05) value		8.42	17.1	3.92	13.8	4.46	15.9

Figures along a column followed by the same letter are not significantly different

Table 4: Effect of nematodes on the mean fresh shoot weights and % reductions in weight of the African indigenous leafy vegetables grown under greenhouse conditions in season two.

Parameter		Plant species					
		Amaranth (*Amaranth hybridus*)	Cowpea (*Vigna unguiculata*)	Sun hemp (*Crotalaria juncea*)	Jute Mallow (*Corchorus spp*)	Spider plant (*Cleome gynandra*)	African night shade (*Solanum nigrum*)
Plant shoot weight(gm)	Inoculated	125.4 a	43.3 a	78.7a	62.7a	85.1a	83.9a
	Non inoculated	129.6a	56b	82.7a	74.8b	88.3a	107.3b
	% reduction	3.3	22.7	5	16.9	3.7	21.8
LSD(p≤0.05) value		4.32	11.35	4.2	9.1	3.56	16.7

Figures along a column followed by the same letter are not significantly different

Dry shoot weight differed significantly (P≤0.05) between the vegetable species. African night shade suffered the greatest dry shoot weight reduction of 47.9 % when innoculated with root knot nematodes followed by jute mallow (42.3 %), cow pea (38.2 %), sun hemp (28.1 %), spider plant (13.3 %) and amaranth (10.8 %) in that order (Figure 1). A similar trend in the dry shoot weight reduction was observed in season two (Figure 2).

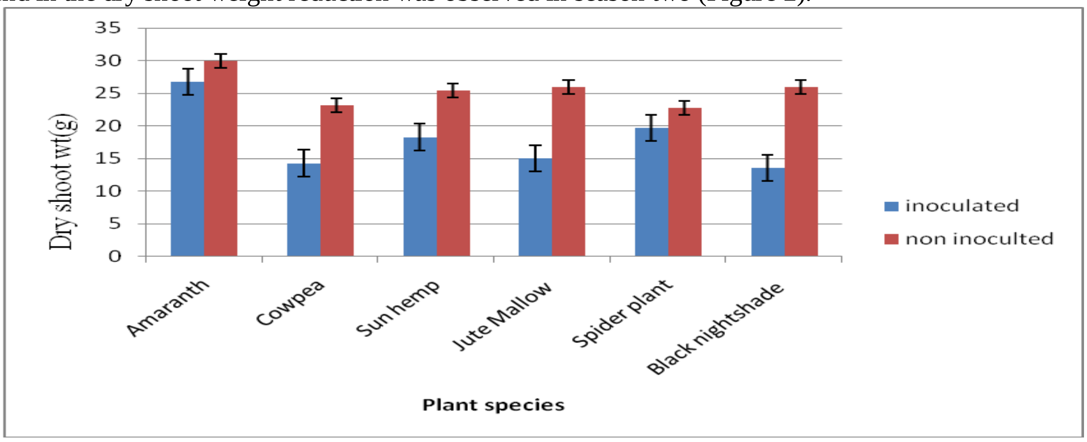

Figure 1: Effect of root knot nematodes infestation on dry shoot weight for selected African indigenous vegetables (Season 1)

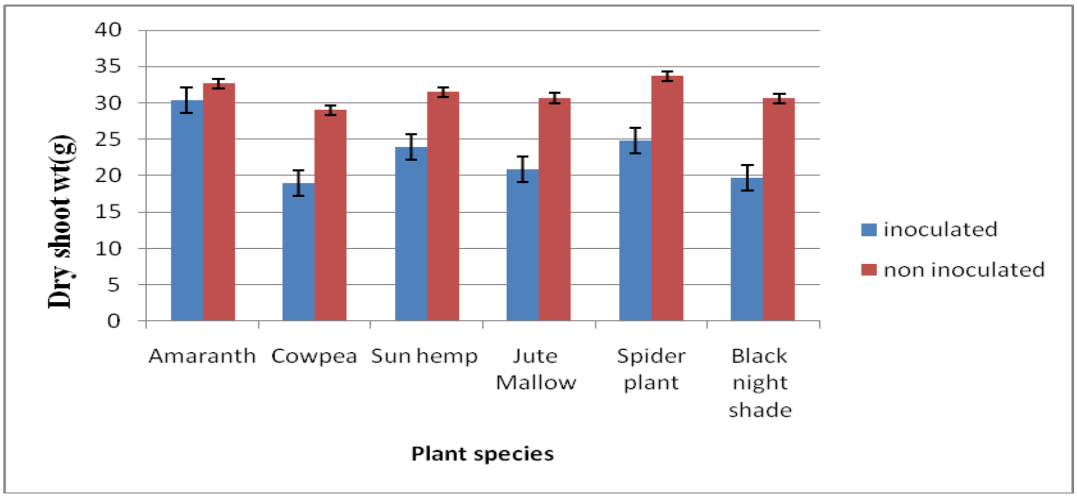

Figure 2: Effect of root knot nematodes infestation on dry shoot weight for Africa indigenous vegetables (Season 2)

The African indigenous leafy vegetable species inoculated with root knot nematodes formed galls of variable sizes. The galling index, egg mass index and juvenile stage two populations differed significantly ($P \leq 0.05$) among treatments (Table 5). The highest galling index rating was observed in the African nightshade at an average of 7 followed by jute mallow (6.7), cowpea (6.3), spider plant (3), sunhemp (2) and amaranth (1.7). African night shade, cowpeas and jute mallow had the highest egg mass indices each with a mean of 3.7 while the least egg mass index of 1 was recorded in amaranth (Table 5).

The three crops, African night shade, cowpea and jute mallow also had high nematode log transformed counts with mean averages of 8.85, 8.42 and 8.42, respectively (Table 5). The least root knot nematode population was recorded in sun hemp at 6.03 and did not differ significantly from 6.45 that was recorded in amaranth (Table 5). The spider plant and sun hemp recorded relatively low rating for galling, egg mass indices and root knot nematode population which were recovered from the soils around the root area (Table 5). The most resistant plant species to the root knot nematodes under greenhouse condition was amaranth with mean galling index of 1.7, egg mass index of 1.0 and second stage juvenile population log transformed mean of 6.45 in both seasons (Table 5 and 6). Egg and galling indices and second-stage juvenile numbers were comparable in Amaranth and sun hemp while the African night shade had the highest egg mass, galling indices and root knot nematode populations.

Table 5: Galling index, egg masses and number of Juveniles observed in the soil and the plant roots for nematode inoculated crop species under greenhouse conditions in season one.

	Crop Variety	Egg mass index (1-5)	Galling index (1-10)	J2 /200cm3 (log_2x)	Reaction
1	Amaranth (*Amaranth hybridus*)	1.0a	1.7a	6.45a	Resistant
2	Cowpeas (*Vigna unguiculata*)	3.7b	6.3b	8.42b	Susceptible
3	Sun hemp (*Crotalaria juncea*)	1.7a	2a	6.03a	Resistant
4	Jute mallow (*Corchorus spp.)*	3.7b	6.7b	8.42b	Susceptible
5	Spider plant (*Cleome gynandra*)	1.7a	3a	6.78a	Resistant
6	African nightshade (*Solanum nigrum)*	3.7b	7b	8.85b	Susceptible
	LSD value ($P \leq 0.05$)	0.92*	0.79*	0.27*	
	Cv%	19.8	9.8	2	

*significance difference ($p \leq 0.05$)
Galling index score (1-10) where 1-3 = resistant and > 3= susceptible,
Egg mass index the plant roots score of 1-5 where 1 = resistant and 5= susceptible,
J2-Second stage juveniles populations recovered from the soil in $200cm^3$,
Log-Logarithm.

Table 6: Galling index, egg masses and number of Juveniles observed in the soil and the plant roots for nematode inoculated crop species under greenhouse conditions season two.

	Crop species	Egg mass index	Galling index	J2(p/$200cm^3$) (log_2x)	Reaction
1	Amaranth (*Amaranth hybridus*)	1.0a	1.3a	6.54a	Resistant
2	Cowpeas (*Vigna unguiculata*)	3.7b	7b	8.49b	Susceptible
3	Sun hemp (*Crotalaria juncea*)	1.0a	1.7a	6.02a	Resistant
4	Jute mallow (*Corchorus spp.)*	3.7b	6.3b	8.38b	Susceptible
5	Spider plant (*Cleome gynandra*)	1.7a	2.7a	6.92a	Resistant
6	African nightshade (*Solanum nigrum)*	3.7b	7.7b	8.78b	Susceptible
	[1]LSD value($p \leq 0.05$)	0.79*	0.92*	0.26*	
	[2]Cv%	17.8	11.4	1.9	

*significance difference ($p \leq 0.05$)
Galling index score (1-10) where 1-3 = resistant and > 3= susceptible,
Egg masses index in the plant roots score of 1-5 where 1 = resistant and 5= susceptible,
J2-Second stage juveniles populations recovered from the soil in $200cm^3$, Log-Logarithm.

4. Discussion

This study has demonstrated that the African indigenous vegetables tested had varied reactions to the root knot nematodes. Amaranth was the most resistant vegetable to the root knot nematodes and could be cultivated in areas where the pathogenic nematodes are endemic. This would ensure sustainable food, income and nutrition security among rural and urban households. The African night shade can be used as a susceptible control in experiements evaluating for resistance to the root knot nematodes. Stunted growth, reduced plant height and vigour in the inoculated vegetables were associated with the root knot nematode infestation. These results compare to those of Mcsorley *et al.* (2004), who reported suppressed plant growth on crops that host root knot nematode. The presence of galls on the roots of susceptible varieties such as the cow pea and African night shade was responsible for stunted growth and wilting of the plants. Galls on the plant roots interfere with nutrients and water absorption leading to discoloration of the leaves displaying symptoms that resemble those of nutrient deficiencies (Atkins *et al.*, 2004). The height of amaranth, sun hemp and spider plant did not differ significantly with non-inoculated controls indicating some levels of resistance to the root knot nematode infestation. These findings compares to a greater extent with those by Nchore *et al*. (2011), who reported less damage on amaranth and more damage on African night shade by root knot nematodes while working on these AILVs in Kisii and Transmara districts of Kenya. Cowpea, jute mallow and African night shade were highly susceptible to the root knot nematode infestation which resulted to reduced height and suppressed growth. Similar findings on infestation by root knot nematodes on cowpeas crop species have been reported (Mcsorley *et al.*, 2004).

The fresh and dry shoot weights of amaranth, spider plant and sun hemp were high despite the root knot nematode infection implying resistance to the pest. Fresh and dry shoot weights of cowpea, African night shade and jute mallow were relatively low implying susceptibility to root knot nematode infestation. The reduced fresh and dry shoot weight on the susceptible vegetables could be attributed to the fact that root knot nematode infestation interferes with water, minerals and nutrients absorption and translocation thus interfering with photosynthesis. The infected plants become stunted; leaves turn yellow, wilt and eventually die (Wesemael and Moens, 2008).

The high egg and galling mass indices observed in the African nightshade, cowpea and jute mallow implied that these crops were more susceptible to the root knot nematodes compared to amaranth, spider plant and sun hemp. African night shade (*Solanum spp)* was the most susceptible and frequently attacked by root knot nematodes compared to the spider plant (*Cleome spp*) and amaranths (*Amaranthus spp).*Similar findings on African night shade and amaranth have been reported by Nchore *et al*. (2011). Susceptible plants to the root knot nematodes, warm temperatures in the greenhouse and sandy soils which were used during experimentation may have contributed to high numbers of second stage juveniles in the soils inoculated with the root knot nematodes. Karssen *et al.* (2007) observed an increased number of root knot nematodes on sandy soils, where susceptible plants were grown under warm greenhouse conditions.

5. Conclusion

Screening of the six AILVs has shown that they are infested by and react differently to RKNs inoculation. Amaranth was the most resistant vegetable whereas sun hemp and spider plant were mildly resistant. African night shade, cowpea and jute mallow were susceptible to RKNs infection. The infestation stunts crop growth through galling, shortening and deforming the roots and lowers the biomass yield required for consumption. This knowledge will enable the development of effective strategies for RKNs management through crop rotation or intercropping and/or selection of appropriate crop cultivars/species for nematode infested soils.

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