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Evaluation of selected kenaf cultivars for their reaction to *Meloidogyne* species

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ABSTRACT
Kenaf is a fibre crop that has various industrial and environmental uses. However, the productivity of the crop is affected by various pathogens including root-knot nematodes. This study evaluated kenaf cultivars for possible resistance to two species of root-knot nematodes. Eight cultivars were evaluated in both micro-plot and field experiments. In the micro-plot experiment, eight selected cultivars were inoculated separately with 5000 eggs *Meloidogyne incognita* and *M. enterolobii* per plant, in comparison to uninoculated control. The experiment was laid out in a randomized complete block design (RCBD) with five replicates. The field plot was naturally infested with a mixed population of *M. incognita* and *M. enterolobii* and laid out in a split-plot arrangement of RCBD with four replicates. Data were collected on plant height, stem circumference and shoot weight, in addition to galling index, and reproductive factor which were used to assess the host status of the kenaf cultivars. The results show that all the cultivars were susceptible to *M. enterolobii* while six were susceptible to *M. incognita*. All these showed reduced plant height, stem circumference and shoot weight. However, cultivar Ifeken-100 was designated as resistant to *M. incognita* while cultivar Ibadan was tolerant.

Keywords: Agronomy yield, fibre, *Meloidogyne enterolobii*, resistant, tolerant.

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is a member of the family Malvaceae and the third largest fiber crop of economic importance after cotton and jute (Starr and Page, 1990). It is an annual crop originating from Africa and disseminated to Asia and then to northern and central USA (Alexopoulou *et al.*, 2013). It became a crop of attention in Nigeria in the 1960's after two factories were established for its processing (Akubueze *et al.*, 2014).

Kenaf is a non-food fibre that is cultivated for paper pulp, fabrics, building materials, biocomposites, bedding material, carpet base and oil absorbents (Nkaa *et al.*, 2007). It has also been recently considered as a medicinal plant due to the records of its seed oil as a therapeutic for blood pressure and cholesterol management (Alexopoulou *et al.*, 2013). This plant produces fibres similar to hardwoods and softwoods, it can be used as a sustainable replacement for products sourced from trees which take a longer time to replace.

The production of the crop, however, comes with various pest challenges including those caused by plant-parasitic nematodes. Significant

reduction of plant height was observed with field populations of *Meloidogyne incognita* (McSorley and Parrado, 1986). The root-knot and reniform nematodes are also reported to increase significantly in kenaf fields with attendant reductions in productivity of the crop (Robinson *et al.*, 1999a). Increased rates of kenaf seedling death had been recorded in fields with high populations of *M. incognita* while the seedlings that survived grew to yield less dry matter (Tahery *et al.*, 2011). The management of nematodes is, therefore, a key factor in the sustainable production of the crop. One of the viable available options is the use of resistant cultivars. Few resistant cultivars have been identified against yield-limiting nematodes. Some cultivars of malvaceous have been designated as tolerant but the tolerance observed in them appeared to be independent of nematode resistance (Davis and May, 2003). The recent interest in kenaf as a fibre crop and the use of the stem fibres for environmental remediation purposes require that its production be unhampered by constraints. The objective of the study was, therefore, to evaluate selected kenaf cultivars for their reaction to populations of two species of root-knot nematodes.

MATERIALS AND METHODS

The experiment consisted of both field and micro-plot experiments conducted in the Crop garden of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan. Seeds of kenaf were collected from the gene bank of the Genetics Unit, CPEB Department. The eight cultivars of kenaf used for this study were NHC6, NHC7, NHC13, V-400, Cuba 108, Abuja, Ibadan and Ifeken-100.

Micro-plot experiment

Seeds were sown in sterilized soil filled into 20 kg micro-plots at four seeds per pot with two seed sown 20 cm apart. Two weeks after planting, thinning and supplying was conducted to arrive at two separated plants per pot. The plants were inoculated one week later with either 5,000 eggs of *Meloidogyne incognita* or *M. enterolobii* each, while the control plants were not inoculated. Treatments were arranged in randomized blocks (RCBD) representing each treatment to prevent cross-contamination of nematode species. These were replicated five times giving a total of 10 units per treatment. Treatments consisted of the eight cultivars of kenaf and three nematode treatments (inclusive of the control). Plants were irrigated and kept weed-free for the 12-week duration of the experiment.

Plant height, number of leaves and stem circumference were taken at anthesis as well as number of days to anthesis. Plant height was taken from the soil line to the plant meristem using a meter rule while the stem circumference was taken at 10 cm from the plant base using a tape measure. At harvest, shoots were cut off at the soil line and weighed. The shoots were then packaged into paper bags, labeled and dried to constant weight in an oven at 70ºC. Plants were dug out with a narrow spade and placed on a large polythene sheet, and roots were separated from soil. Separate sheets were used per treatment. The roots were washed free of soil, weighted and prepared for nematode extraction. Rhizosphere soil per micro-plot was thoroughly mixed and two 250 cm^3 samples were taken out for extraction.

Roots were evaluated for damage using the gall rating scale of 1-5; where 1= no galls, 2=1-15% of galled root, 3=16-30%, 4=31-60%, 5=61-100% (Claudius-Cole, 2005). After this, roots were chopped into 2-3 cm pieces from which 10 g were weighed out for nematode extraction. *Meloidogyne* eggs were extracted from the roots using the sodium hypochlorite method (Southey, 1986). Nematodes were counted from each sample and used to calculate the number of nematodes in the whole root system. Mobile nematodes were extracted from the measured root samples using the extraction

tray method (Coyne *et al.*, 2007). Nematodes counted from the extract were used to calculate the total number of nematodes in the soil. The nematode counts in roots and soil were then summed to derive the final nematode population (Pf). This value was then used to calculate reproductive factor (RF) which is Pf/Pi, Pi being the initial nematode population. Host status was determined using the rating of (Sasser *et al.*, 1984) where Resistant = GI<2, RF<1; Tolerant = GI<2, RF>1; susceptible = GI<2, RF>1 and Hypersusceptible = GI<2, RF<1.

Field experiment

The field experiment was conducted at the Crop garden of the CPEB Department, located 7°27'01.2"N 3°53'48.3"E, at an elevation of 205 m above sea level. The field plot was naturally infested with mixed populations of *M. incognita* and *M. enterolobii* (Claudius-Cole *et al.*, 2017a). The plot had been previously planted to tomatoes (for how long?) to increase the nematode populations. The field was marked out into plots and six cores were taken per plot and bulked. The bulked soil was thoroughly mixed and a 200 cm^3 sample was teken for nematode extraction. Nematode extraction using the extraction tray method was conducted per sampled plot and the number of nematode per plot estimated. Initial estimated nematode population (Pi) per plot ranged between 120 - 160 J2/ 200 cm^3 of soil. The experiment was a split plot layout fitted into a randomized complete block design with four replications. The plots for control were located in a part of the Crop garden with undetectable root-knot nematode populations and treated with carbofuran 3G at the rate of 10 g/m^2 (10 kg/ha) two weeks prior to planting. The main plot effect was the nematode treatment of which there were two while the sub-plot treatment was the eight kenaf cultivars. Each plot was 0.5 x 5 m rows with kenaf seeds planted at 25 cm spacing within rows and 0.5 cm between rows. Plots were kept weed-free for the 12 week-duration of the trial. Data c were collected from 10 plants per plot, following the same procedures as described for the micro-plot experiment.

Data analysis

Data on nematode counts were transformed using $\sqrt{x+0.5}$. The procedure for the generalized linear model was used for analysis of variance using the SAS version 9.2 statistical package. Significant means were separated using Student Newman Keuls or Fisher's least significant means at 5% level of significance as appropriate. Standard error was used as appropriate to identify significant differences among means on charts.

RESULTS

Kenaf plants inoculated with *Meloidogyne incognita* and *M. enterolobii* showed a reduction in yield parameters of kenaf in micro-plots (Table 1). The results presented in the Table also show similar yield limiting effect of the two root-knot nematode species. For the specific kenaf cultivars in micro-plots, significant differences were observed in the measured parameters among some cultivars inoculated with both root-knot nematode species (Table 2). Plant height was not significantly different between control and inoculated plants of cv Abuja, and NHC13, while cv Cuba, NHC6, NHC7, V-400, and Ibadan had lower ($p \leq 0.05$) plant height when inoculated with both nematode species. However, plant height of cv Ifeken-100 was not significantly different when inoculated with *M. incognita* but was reduced significantly with *M. enterolobii*. Shoot weight was significantly reduced as a result of inoculation with both *M. incognita* and *M. enterolobii* for cv Abuja, NHC13, NHC6, and NHC7 but for cv V-400 and Ifeken-100 significantly reduced shoot weight was observed only for *M. enterolobii*-inoculated plants. With reference to the stem

Table 1: Plant height, shoot weight and stem circumference of kenaf plants inoculated with *Meloidogyne* species in micro-plot trials

Treatment	Plant height (cm)	Shoot weight (g)	Stem circumference (cm)
Control	147.9a	109.3a	16.4a
M. incognita	125.1b	57.3b	15.0b
M. enterolobii	128.2b	64.8.7b	14.8b

Values are means of five replicates with 2 plants per micro-plot.
Means with the same letter in a row are not significantly different using Student Newman Keuls at p≤ 0.05

Table 2: Effect of *Meloidogyne* spp. on yield parameters of kenaf cultivars in a micro-plot experiment

Cultivar	Plant height (cm)			Shoot weight (g)			Stem circumference (cm)		
	Control	*M. incognita*	*M. enterolobii*	Control	*M. incognita*	*M. enterolobii*	Control	*M. incognita*	*enterolobii*
Abuja	47.25	44.00	42.68	282.75	162.00*	155.99*	4.68	4.11	3.89*
Cuba	61.25	52.25*	50.68*	150.50	145.50	140.27	4.25	4.78	4.01
NHC13	54.00	50.75	49.23	115.75	87.75*	94.07*	5.38	4.93	4.65*
NHC6	105.25	77.00*	74.69*	132.50	51.75*	62.58*	3.79	3.01	2.80*
NHC7	68.00	38.50*	41.35*	186.67	155.25*	136.70*	3.00	3.91	3.46
V-400	45.00	31.25*	36.31*	211.25	186.75	179.56*	3.94	3.45	3.20
Ibadan	66.13	52.38*	43.81*	128.13	116.63	103.11	3.92	3.06	2.39*
Ifeken-100	56.75	53.63	47.26*	159.58	123.50	91.70*	3.11	2.50	2.31
LSD	13.53	9.62	8.31	39.83	31.71	27.77	0.71	0.61	0.53

Values are means of five replicates with 2 plants per microplot.
* = significant difference among means in a row for each parameter using LSD at P≤0.05.

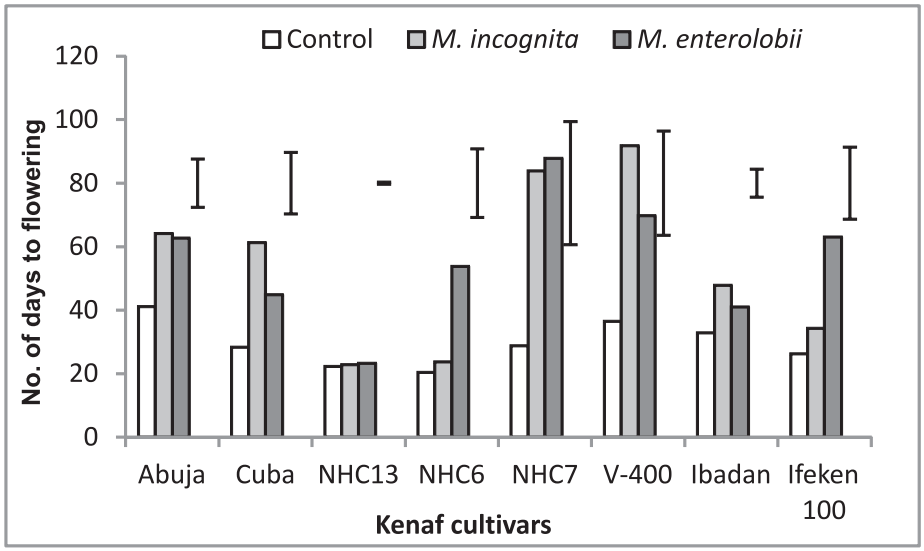

Figure 1: Influence of root-knot nematodes on the number of days to flowering
Bars = Standard error

circumference, only *M enterolobii* caused a significant reduction in cv Abuja, NHC 13, NHC6, and Ibadan. There was significant delay in flowering between the control plants and the *Meloidogyne*-inoculated plants (Figure 1). However, for the cultivar NHC13 there was no significant difference in number of days to flowering in the control compared to the inoculated plants. Flowering in NHC7 and V-400 was delayed by between 55-58 days(over 100%) with both *Meloidogyne* spp. compared to the control. Significant delay in number of days to flowering was observed in NHC6 (30 days) and Ifeken-100 (28 days) kenaf cultivars when inoculated with *M. enterolobii* compared to *M. incognita*.

Among the cultivars, Ifeken-100 and Ibadan had significantly lower root galling compared to other cultivars with a similar trend observed for the number of nematodes recovered from roots and soil and the reproductive factor (Table 3). Reproductive factor of both root-knot nematode species varied with cultivar. *Meloidogyne incognita* reproduced significantly more in NHC6 and Cuba compared to *M. enterolobii* while, *M. enterolobii* reproduced significantly more in NHC7, V-400 and Ifeken-100 than *M. incognita*. Comparison between the galling induced by both nematodes shows that *M. incognita* induced more severe but not significant galling compared to *M. enterolobii*. This was however not the case with Ibadan and Ifeken-100 that had more galls in plants inoculated with *M. enterolobii* compared to those inoculated with *M. incognita.* The reproductive factor of *M. incognita* in Ifeken-100 was <1 whereas that of *M. enterolobii* was significantly higher at 3.59 in the microplot experiment. Kenaf cultivar Ifeken-100 was designated as resistant to *M. incognita* with galling index < 2 and RF <1 while Ibadan was designated as tolerant to *M. incognita* with galling index < 2 and RF > 1. However, all the cultivars were designated as susceptible to *M. enterolobii*.

The tallest plants among cultivars growing in the control field plots were Cuba, Ibadan and Ifeken-100. Stem circumference was wider in cultivar NHC7 and Abuja among all cultivars in the control plots. The cultivars with the highest shoot weight in the control plots were Abuja, NHC7, V-400 and Ibadan (Table 4).

Table 3: Galling index and populations of *Meloidogyne incognita* and *M. enterolobii* on selected kenaf cultivars

	Galling index		Total no. of nematodes		Reproductive factor		Host status	
Cultivar	*M. incognita*	*M. enterolobii*	*M. incognita*	*M. enterolobii*	*M. incognita*	*M. enterolobii*	*M. incognita*	*M. enterolobii*
Abuja	4.50	3.95	121.39	103.79	11.79	8.62	S	S
Cuba	4.75	4.08	127.75	118.06	13.06*	11.15	S	S
NHC13	4.00	3.70	126.69	110.72	12.84	9.81	S	S
NHC6	3.25	3.33	318.17*	115.26	40.99*	10.63	S	S
NHC7	4.75	4.08	122.44	165.99	11.99	22.04*	S	S
V-400	5.00	4.20	115.29	153.55	10.63	18.86*	S	S
Ibadan	1.80	2.00	81.67	97.04	3.34	5.53	T	S
Ifeken 100	1.18	2.59*	28.12	67.03*	0.63	3.59*	R	S
LSD	1.03	0.57	59.44	22.33	18.34	4.32		

Values are means of five replicates with 2 plants per micro-plot. * = significant difference among means in a row for each parameter using LSD at P≤0.05. Host status rating - Resistant = GI<2, RF<1; Tolerant = GI<2, RF>1; susceptible = GI>2, RF>1 and Hypersuceptible = GI<2, RF<1.

In the field experiment, plant height was also generally lower in *Meloidogyne*- infested plots compared to the control plots (Table 4), with significantly lower height in cultivars Abuja, Cuba, NHC13, V-400 and Ibadan. Stem circumference of cultivars NHC6, NHC7, and Ibadan was significantly reduced in plots infested with root-knot nematodes in comparison to the control plots. The shoot weight was also negatively affected by root-knot nematodes and was significantly reduced in infested plots with Abuja, NHC7, V-400 and Ibadan.

Some damage, evidenced in the presence of a few galls was observed in the control plots but the galls were significantly fewer compared to observation from plots that were infested with *Meloidogyne* spp (Table 5). As anticipated, nematode populations and reproductive factor were significantly higher in the infested plots compared to the control plots. Among the cultivars in the nematode-infested plots, significantly lower damage was observed in Ifeken-100 and Ibadan with a similar trend also observed for nematode populations and reproductive factor. Following the host status rating, kenaf cultivar Ifeken-100 was designated as resistant with galling index < 2 and RF <1 while cultivar Ibadan was designated as borderline between susceptible and tolerant with galling index = 2 and RF >1 (Table 5).

Table 4: Effect of field populations of *Meloidogyne* spp. on yield of selected kenaf cultivars

Cultivar	Plant height (cm)		Stem circumference (cm)		Shoot weight (g)	
	Control	*Meloidogyne* infested field	Control	*Meloidogyne* infested field	Control	*Meloidogyne* infested field
Abuja	165.38	125.15*	7.20	6.32	327.38	279.83*
Cuba	214.38	160.90*	6.54	7.35	226.25	200.45
NHC13	189.00	138.15*	8.28	7.58	168.13	159.20
NHC6	163.13	146.90	5.83	4.32*	159.76	149.58
NHC7	170.00	155.07	7.62	4.56*	289.28	226.16*
V-400	157.50	123.65*	6.06	5.32	327.25	256.45*
Ibadan	201.46	140.53*	6.02	4.71*	305.22	266.74*
Ifeken 100	198.63	171.98	4.24	5.39	263.19	237.03
LSD	15.10	12.08	1.10	0.94	58.38	34.34

* = significant difference among means in a row for each parameter using LSD at P≤0.05.

Table 5: Damage and population of *Meloidogyne* spp on kenaf cultivars in field plots

Cultivar	Galling index		Total no. of nematodes		Reproductive Factor		Host Status
	Control	*Meloidogyne* infested field	Control	*Meloidogyne* infested field	Control	*Meloidogyne* infested field	
Abuja	1.33	3.31*	1.61	219.37*	0.43	10.20*	S
Cuba	1.00	3.34*	1.67	443.35*	0.28	19.66*	S
NHC13	1.67	4.03*	0.07	240.33*	0.06	5.78*	S
NHC6	1.00	3.62*	0.19	385.81*	0.06	14.88*	S
NHC7	1.00	4.44*	1.50	441.23*	0.13	19.47*	S
V-400	1.33	4.58*	1.44	553.08*	0.16	30.59*	S
Ibadan	1.00	2.04*	1.37	103.66*	0.19	1.87*	S/T
Ifeken-100	1.00	1.82	1.30	34.50*	0.23	0.55	R
LSD	0.18	0.62	0.46	129.74	0.09	7.35	

* = significant difference among means in a row for each parameter using LSD at P≤0.05.
Resistant = GI<2, RF<1; Tolerant = GI<2, RF>1; susceptible = GI>2, RF>1 and Hypersusceptible = GI<2

DISCUSSION

In both the micro-plot and field experiments, yield parameters were generally negatively affected when kenaf plants were grown in soil with both species of nematode. Although their reaction varied with the specific parameter measured and the cultivar assessed. For kenaf, the parameters for yield are based on characteristics of the stem which is the economic part of the plant referred to as agronomic yield while the seed production is referred to as seed yield. Of importance are the height of the plant, the width of the stem and the density of the fibres with are correlated with weight (Agbaje *et al.*, 2011). Any stress therefore that interferes with these parameters would lead to agronomic yield reduction. The reduced plant height observed in this study was also reported by McSorley and Parrado (1986). In their study, the authors reported that plant height was negatively correlated with nematode density. They also showed that the tolerance level of the kenaf varieties they used was 8 galls/ egg masses per plant. The implication is that in a susceptible cultivar more than 8 galls/ egg masses, corresponding to a galling index of 2, is sufficient to reduce plant height. In this study it was observed that there was a significant stunting in most of the cultivars in response to infection by both root-knot nematode species. Nematode infection on kenaf is reported to result in increased internodes and thinner stalks (Tahery *et al.*, 2011) which is confirmed in this study with the stalks in infected plants being thinner both in microplot and field trials. Significant reduction in stalk thickness was also more evident in *M. enterolobii*-infested microplots. The sensitive cultivars with respect to this parameter were Abuja, NHC6 and Ibadan. Like other paramenters, the shoot weights of inoculated plants were lower than those of control plants in the microplot experiment, but differences varied with the species of root-knot nematode involved. Cultivars that had significant reduction in shoot weight with *M. incognita* also had the same response with *M. enterolobii*. Reduction in shoot weight was more pronounced with *M. enterolobii* than *M. incognita*. This further shows that there is a greater sensitivity of the kenaf cultivars to the *M. enterolobii*. For example, Ifeken had a non significant shoot weight reduction with *M. incognita* whereas the shoot weight with *M enterolobii* was significantly lower than both the control and *M. incognita*-inoculated plants. Adegbite *et al.* (2008) similarly reported that in Ifeken-100 there was no significant reduction in shoot weight when incolulated with *M. incognita*. *Meloidogyne enterolobii* has a wide host range and has been reported as parasitic on plants from many families including Curcubitaceae, Chenopodiaceae, Fabaceae, Myrtaceae, Solanaceae, and Umbelliferae (Rodriguez *et al.*, 2003).

The host status based on the galling index and reproductive factor show that six out of the eight cultivars were susceptible to *M. incognita*. The other two, Ibadan and Ifeken-100 were designated tolerant and resistant respectively. With *M. incognita,* Ibadan cultivar showed mild to moderate galling, Ifeken-100 showed no to mild galling and the other cultivars were severely galled. The reaction of Ifeken-100 to *M. incognita* in the current study is similar to the findings of Adegbite *et al.* (2005). Other authors have also reported that kenaf cultivars are susceptible to *M. incognita, M. javanica* and *Rotylenchulus reniformis* (Lawrence and McLean, 1992; Barillas *et al.*, 1993; Robinson *et al.*, 1999b). Severe galling was observed in the roots of all the cultivars in response to *M. enterolobii*. The presence of a mixed population of *Meloidogyne* spp. including *M. enterolobii* in the field plots appeared to compromise the resistance/tolerance observed in cultivars Ibadan and Ifeken-100. The wide distribution of *M. enterolobii* combined with the ability of the nematode species to parasitize crops with resistance to other *Meloidogyne* spp.

demonstrate the potential economic impact of *M. enterolobii* on the agricultural industry (Brito *et al.*, 2007). The nematode has been found to be severely parasitic on tomatoes, pepper (Claudius-Cole *et al.*, 2017b) and yams (Kolombia *et al.*, 2016). The presence of this species of root-knot nematode in field populations presents new challenges in using resistance to manage root-knot nematodes. This implies that other methods should be combined with resistance to achieve effective nematode management in kenaf.

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