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EVALUATION OF KENAF (*HIBISCUS CANNABINUS*) ACCESSIONS FOR RESISTANCE TO *MELOIDOGYNE INCOGNITA*

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ABSTRACT

The breeding of pest and disease resistant varieties is one of many strategies of improving kenaf production, but it is vital to identify cultivars with resistance prior to breeding for resistant varieties. In 2013, ten kenaf accessions were screened for resistance to *Meloidogyne incognita* in a potted experiment laid out in completely randomized design pending field trials. Seeds of kenaf accessions were sown into polypots filled with 10 kg of steam-sterilized soil. Each kenaf seedling was inoculated with 10,000 eggs of *M. incognita* at three weeks after sowing. At nine weeks after inoculation (WAI), data were collected on plant height, number of leaves, gall index, final nematode population (P_f), and reproductive factor (RF) and analyzed with ANOVA. Host resistance status was assigned to the kenaf accessions using Canto-Saenz. NHC-16 had the highest height of 112.2 cm, whereas NHC-40 had the highest number of leaves (41.6). Best growth was observed in NHC-16, NHC-40 and NHC-400. The lowest P_f and RF of 61760 and 6.1 respectively were obtained in NHC-28. All the kenaf accessions were all susceptible to *M. incognita*, but NHC-28 showed slight susceptibility. Pending the discovery of more resistant kenaf genotypes, NHC-28 is therefore recommended for field trials.

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.; Malvaceae) is one of the three largest fibre crop of economic importance (Keshk *et al.,* 2006). Kenaf is reported to have originated from Africa and being cultivated in several parts of the world (Dempsey, 1975; Webber, 1996; LeMahieu *et al*., 2003). It is commonly cultivated for both food and fibre in West Africa (Adegbite *et al.,* 2005). Kenaf has been used as a cordage crop to produce twine, rope and sackcloth for over six millennia (Dempsey, 1975). Commercially, kenaf is cultivated purposely for pulping and paper making, oil spills bioremediation, livestock nutrition and bio-degradable packaging materials (Wildeus *et al*., 1995; Cheng, 2001; Adeniyan *et al*., 2014). In spite of the outstanding contributions of kenaf to man's existence, it suffers notable damages from pests and pathogens. Such pests include insects, fungi, bacteria, plant-parasitic nematodes (PPNs), amongst others (Adeniji, 1970; Adegbite *et al.,* 2005). Earlier researches have demonstrated that kenaf is susceptible to plant-parasitic nematodes, especially root-knot nematodes (RKNs), *Meloidogyne* species, such as *M. incognita, M. arenaria* and *M. javanica* (Summers and Seale, 1958; Adeniji, 1970; Minton *et al.,* 1970; McSorley and Parrado, 1986; Adegbite *et al.,* 2005). Symptoms of root-knot nematode infection on kenaf include yellowing of leaves, defoliation, stunted growth, large root galls, poor dry matter yield and eventual death of the plants before they reach maturity (Summers and Seale, 1958; Dempsey, 1975). Based on population density of nematode in the soil, kenaf yield could be reduced by 32-67% (Nieschlag *et al.,* 1961; Sasser *et al.,* 1984; Lawrence and McLean, 1992). Management of PPNs by host resistance has shown promise because it is at no extra cost to the farmers, and it reliefs the environmentalists extra concern for safety (Atungwu *et al*., 2008). Resistant cultivars can produce the most dramatic increase in yield of many crops and appear to hold the solution to most nematode problems (Luc *et al.,* 2005). It is the most cost-effective and sustainable management tactic for preventing root-knot nematodes damage and reducing growers' losses (Khan, 1994). Resistant crop cultivars have comparatively better crop yield than susceptible crop cultivars (Luc *et al.,* 2005). In plant nematology, relative to a disease-susceptible plant, plant disease resistance is often defined as the ability of a plant to inhibit the reproduction of a nematode species relative to reproduction on a plant lacking such resistance (Friedman and

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Baker, 2007). After infection by the same nematode strain at similar inoculum levels, a gradation of quantitative differences in disease resistance is possible between plant lines or genotypes (Lucas, 1998). Adegbite *et al.* (2005) reported Ifeken 100 and 400 as resistant and poor host respectively of *M. incognita* out of ten kenaf cultivars screened against *M. incognita.* Some studies have produced estimates of root-knot nematode effect on kenaf (McSorley and Parrado, 1986; Adegbite *et al.,* 2005), but there is still insufficient data on effect of root-knot nematode on growth and productivity of many recently released kenaf genotypes, especially in Nigeria for cultivation in the Niger Delta and Southeast regions. On the other hand, previous researches had not provided a comprehensive comparison among large number of kenaf varieties with a view to selecting resistant varieties to *M. incognita* for cultivation in southeastern Nigeria. This, if done will promote good growth, yield and proper management of root-knot nematodes on kenaf using crop rotation. In this study, ten recently released kenaf accessions in Nigeria were screened for resistance to the root-knot nematode (*Meloidogyne incognita*) prior to their introduction to farmers for cultivation in the Niger Delta region of Nigeria for various uses, especially as a bioremediation agent for cleaning hydrocarbon polluted sites.

MATERIALS AND METHODS

Experimental site/ laboratory

This investigation was conducted in the rainy season from June to August, 2013 at the Research Farm and the Laboratory of Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt, Port Harcourt Rivers State. The Research Farm lies on latitude 04˚538. 3^{11} N and longitude 0.06^{o} 54.38. 0^{11}E in Southern Nigeria. Other laboratory studies were carried out at the Nematology Research Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Oyo State.

Source of kenaf seeds

The seeds of ten kenaf accessions were obtained from the Kenaf Genebank at the University of Ibadan, Ibadan, Oyo State, Nigeria. The accessions were; NHC 14, NHC 16, NHC 20, NHC 22, NHC 25, NHC 28, NHC 30, NHC 31, NHC 40 and NHC 400.

Soil sterilization

Top soil (loamy sand) was collected from the Research Farm, Department of Crop and Soil Science, University of Port Harcourt and poured into a drum. Water was added to the soil then steamed-sterilized for four hours at 80^{o}C after which the soil was allowed to cool and kept in bags for three weeks to regain stability.

Source and culture of *Meloidogyne incognita*

Meloidogyne incognita used in the investigation was originally isolated from *M. incognita* culture plot maintained with *Celosia argentea* at the National Horticultural Research Institute (NIHORT). *Meloidogyne incognita* was later inoculated into two-week old *Celosia* seedlings grown on steam-sterilized sandy-loam soil in pots and allowed to reproduce in the roots of *Celosia argentea* plants for eight weeks.

Preparation of experimental pots

Ten-litre black polyethene bags perforated with 15 holes each to allow for even drainage served as pots. Each pot was filled with 10 kg of steam-sterilized sandy-loam top soil prior to sowing and arrangement.

Sowing, experimental design and thinning of kenaf seeds before inoculation

Three seeds of each kenaf accession were planted in a polyethene bag (diameter 22 cm and depth of 30 cm) containing 10 kg steam-sterilized soil. Each kenaf accession was replicated four times and arranged using completely randomized design in a sheltered space with tile flooring on the ground floor of the Department of Crop and Soil Science, University of Port Harcourt. This was done in order not to introduce nematode into the environment. The plants were later thinned to one plant per bag at two weeks after sowing (WAS).

Inoculation of kenaf seedlings with *M. incognita*

Three weeks after sowing, each kenaf seedling was inoculated with egg suspension of *M. incognita* containing 2,500 eggs per ml. Four ml of egg suspension containing 10,000 eggs of *M. incognita* was drawn with a 5 ml syringe and released into four holes of a depth of 4 cm each made around the plant roots after which the holes were covered with soil.

Data collection

Post-inoculation growth performance of each kenaf accession was evaluated by measuring plant height (cm) and number of leaves was visually counted at weekly intervals until the experiment was terminated at nine weeks after inoculation (WAI). At the end of the experiment, the plants were harvested and fresh shoot weight (g) determined using an electronic balance. The root system of each kenaf accession was carefully dug, rinsed with water and then rated for galls using the method of Osunlola (2011), where: 0 = no gall; 1 = 1-20% of the root system galled; 2= 21-40% of the root system galled; 3 = 41-60% of the root system galled; 4= 61-80% of the root system galled; and 5 = 81-100% of the root system galled. After the gall rating, the entire root system of each plant were cut into 1-2 cm pieces and shaken vigorously in 0.5 % sodium hypochlorite (NaOCL) solution to extract the eggs (Hussey and Barker, 1973). The content was then poured into 200 mesh sieve nested over 500 mesh sieve. The 200 mesh sieve retained the roots and the debris, while the 500 mesh sieve retained the eggs which were later rinsed with water into a beaker using a wash bottle. The content was allowed to settle and the supernatant decanted. An aliquot of 1 ml of the egg suspension was placed in a Doncaster dish (Doncaster, 1962) and counted with a tally counter under a stereomicroscope.

The second-stage juvenile (J_2) populations were also estimated from 250 ml soil obtained from each pot using the pie-pan method (Whitehead and Hemming, 1965). The infected soil was thoroughly mixed together and sieved to remove stones and debris, after which 200 ml of sieved soil sample was placed on a facial tissue in a plastic sieve and water added to the extraction plates. The set-up was allowed to stand for 48 hours after which the sieves were removed. The suspension in the extraction plate was poured into a beaker, allowed to settle and then the water was gently decanted. The juvenile population was estimated using a stereomicroscope. The total number of J_2 in the soil per pot was extrapolated from the number of second-stage juveniles. The population of J_2 in the soil per pot was added to the number of eggs extracted from the roots to obtain the final nematode population (Pf). The host efficiency was determined by the reproductive factor (Pf/Pi); where Pf (final nematode population) and Pi = 10,000 eggs, the initial population density. A reproduction factor of >1 indicates an increase in nematode reproduction where an RF factor of <1 implies no increase in reproduction. The final assessment of resistance of various cultivars was based on Canto-Saenz's host designation scheme (Sasser *et al.*, 1984).

Plants with GI (Gall index) > 2 are defined as either susceptible (RF >1) or hyper susceptible (RF ≤ 1); plants with GI ≤ 2 are classified either resistant (RF ≤ 1) or tolerant (RF > 1). Besides the parameters taken above, the roots were inspected for presence and absence of necrotic lesions and root density.

Statistical analysis

Data collected were analyzed using analysis of variance and means separated with Least Significant Difference (LSD) test at 5% level of probability using the Statistical Analysis Systems (SAS, 2009).

RESULTS

Effects of *Meloidogyne incognita* infection on growth of ten kenaf accessions

At inoculation (AI), NHC-31 had the highest height (21.4 cm) followed by NHC-28 (20.6 cm) and NHC-22 (20.3 cm) (Fig. 1).

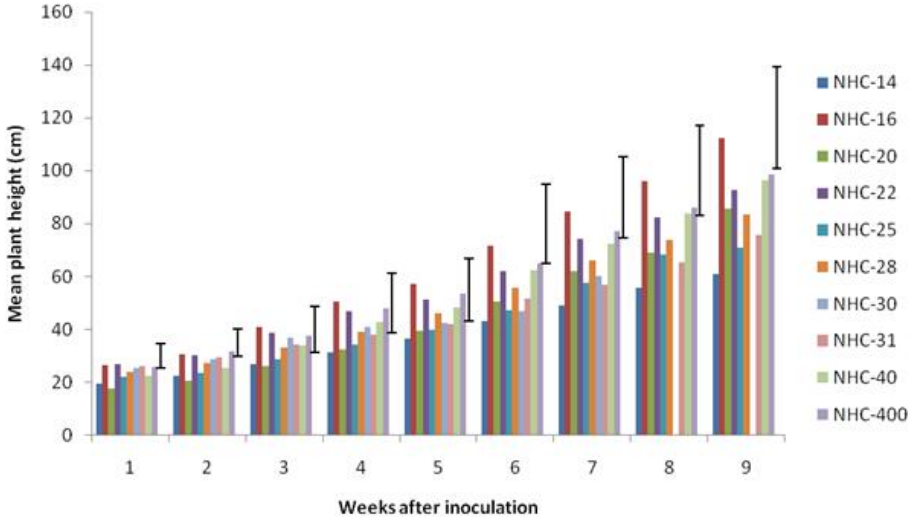

Fig. 1. Effects of *Meloidogyne incognita* on mean plant height (cm) of ten kenaf accessions

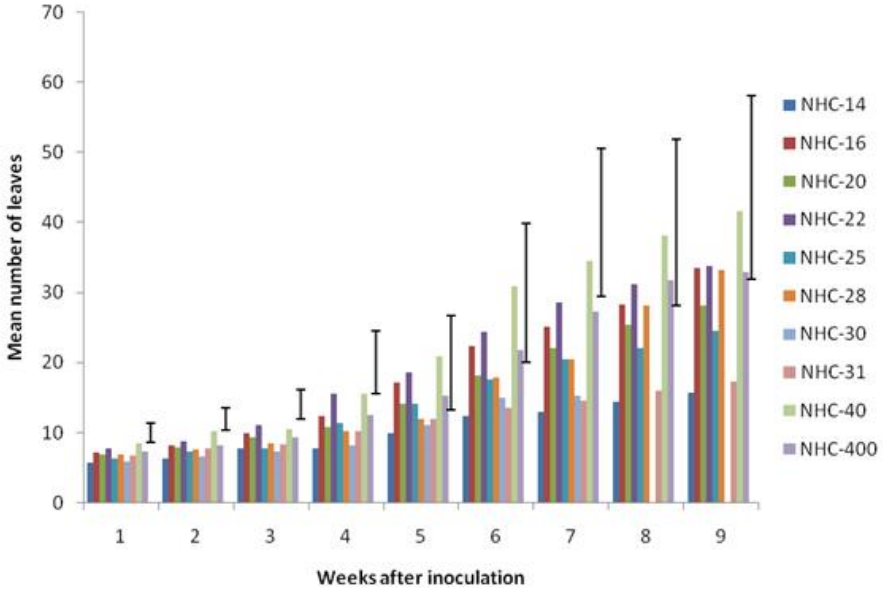

Fig. 2. Effects of *Meloidogyne incognita* on mean number of leaves of ten kenaf accessions

There was a gradual increase in the height of kenaf plants after inoculation with NHC-16 showing the best height of 112.2 cm nine weeks after inoculation (WAI). This was significantly higher than the height of NHC-400 which was 98.7 cm at nine WAI. Other accessions that had relatively good growth in height were NHC-40 (96.3 cm) and NHC-22 (92.8 cm). By the eight week after inoculation, all NHC-30 plants had died so no data was taken beyond the seventh WAI. NHC-40 had the highest number of leaves (41.6), followed by NHC-22 (33.8), NHC-16 (33.6), NHC-28 (33.2) and NHC-400 (33.0), respectively (Fig. 2). On the contrary, NHC-14 had the least number of leaves (15.8), followed by NHC-31 (17.4). Generally, there was an increase in the number of leaves although the rate of leaf production and size varied among the accessions. NHC-30 plants were dead by the eight week after inoculation but by the seventh week it was among the three accessions (NHC-14, NHC-30 and NHC-31) with the least number of leaves. The fresh shoot weight (g) of the kenaf accessions screened is presented in Table 3.

Table 3. Effects of *Meloidogyne incognita* on fresh shoot weight of ten kenaf accessions

Accessions	Fresh shoot weight (g)
NHC-14	37.9
NHC-16	58.1
NHC-20	54.6
NHC-22	75.7
NHC-25	54.9
NHC-28	65.1
NHC-30	0
NHC-31	64.3
NHC-40	77.0
NHC-400	71.3
LSD(P≤0.05)	20.9

0.0- All plants shriveled, died at 8 WAI and no data collected.

Since NHC-30 plants had died before termination of the study, no data was taken. NHC-14 had the least fresh shoot weight (37.9 g) and it was less than the shoot weights for NHC-20 (54.6 g), NHC-25 (54.9 g) and NHC-16 (58.1 g). There was no difference in the shoot weights of NHC-20, NHC-25 and NHC-16. The highest shoot weight was recorded for NHC-40.

Effects of *Meloidogyne incognita* infection on gall index and nematode population of ten kenaf accessions

At nine WAI, NHC-14 and NHC-25 were heavily galled having the highest gall index of 5.0 which was not significantly different from NHC-20 having 4.8 as the gall index (Table 4). Generally, NHC-14 had the highest nematode population (NP) of 550,040 and it highly supported the reproduction of nematode having the highest reproductive factor (RF) of 55.0. This was not significantly different from NHC 400 having a NP and RF of 446,520 and 44.7 respectively (Table 4).

Host resistance designation of the ten kenaf accessions to *Meloidogyne incognita*

Based on Canto-Saenz (Sasser *et al*., 1984) resistance ratings, the ten kenaf accessions were susceptible to *Meloidogyne incognita* (Table 5). This shows that these kenaf plants have gall index > 2 and reproductive factor (RF) >1.

Table 4. Effects of *Meloidogyne incognita* on mean gall index, egg population, second stage juveniles (J_2), final nematode population (P_f) and reproductive factor (RF) of ten kenaf accessions

Accessions	Gall index	Egg population	J_2	P_f	RF
NHC-14	5.0	549,000	1,040.0	550,040	55.0
NHC-16	3.4	112,680	400.0	113,080	11.3
NHC-20	4.8	183,240	1,560.0	184,800	18.5
NHC-22	3.8	317,520	1,300.0	318,700	31.9
NHC-25	5.0	307,920	1,280.0	309,120	30.9
NHC-28	2.4	61,200	560.0	61,760	6.1
NHC-30	0	0	0	0	0
NHC-31	3.0	158,760	1,200.0	159,960	16.0
NHC-40	3.0	144,840	1,120.0	146,046	14.6
NHC-400	3.4	445,440	1,560.0	446,520	44.7
LSD(P≤0.05)	0.8	259,566	378.7	259,705	25.9

0.0- All plants shriveled, died at 8 WAI and no data collected; J_2 = Second-stage juveniles, P_f = Final nematode population; RF = reproductive factor.

Table 5. Canto-Saenz resistance rating of ten kenaf accessions to *Meloidogyne incognita*

Accessions	Gall index	Reproductive factor	Rating
NHC-14	5.0	55.0	Susceptible
NHC-16	3.4	11.3	Susceptible
NHC-20	4.8	18.5	Susceptible
NHC-22	3.8	31.7	Susceptible
NHC-25	5.0	30.9	Susceptible
NHC-28	2.4	6.0	Susceptible
NHC-30*	0	0	Susceptible
NHC-31	3.0	16.0	Susceptible
NHC-40	3.0	14.6	Susceptible
NHC-400	3.4	44.7	Susceptible
LSD(P≤0.05)	0.8	25.9	

* All plants shriveled, died at 8 WAI, indicating the highest susceptibility rating.

General observations

There were leaf spots, leaf drop and chlorosis at the early stage of plant growth. Eight weeks after sowing (WAS), scale insects surrounded by ants where found under the leaves of the plants especially on NHC-14, NHC-16 and NHC-28. These insects may have been responsible for the transmission of viral infections evident as mosaic symptoms and necrotic local lesions. At 12 WAI, wilting was observed on NHC-25 and NHC-22. Besides the galls on the roots, there where necrotic lesions on NHC-14, NHC-16, NHC-20, NHC-22 and NHC-25.

DISCUSSION

The susceptibility of any plant to *M. incognita* depends on the ability of *M. incognita* juveniles to penetrate the roots of the plants and cause the formation of giant cells which appears as knots (galls) on the roots (Chen *et al*., 2004). The presence of root galls on all the ten kenaf genotypes that were screened indicates susceptibility to *M. incognita*. This showed that neither pre- nor post-infective defense mechanisms to restrict or prevent the nematode's reproduction were activated in the kenaf accessions to confer resistance (Huang, 1985). Mechanisms of pre-infection if activated by host plant against *Meloidogyne incognita* should limit penetration of the infective second-stage juveniles (J_2) via pre-existing morphological barriers or the production of substances that repel them from the host plant (Jatala and Russell, 1972; Huang, 1985). Since there were massive galls observed on

most of the kenaf roots, the likelihood of inhibition of formation of feeding sites, prevention or delay of J_2 development and reproduction of the adult female have not taken place in these kenaf accessions after being infected by *M. incognita.* Physiological and molecular processes in post-infection mechanisms might not have been activated in these kenaf accessions screened in response to infection (Huang, 1985; Anwar and McKenry, 2000). The susceptibility of these kenaf accessions to *M. incognita* was also evident in the high population and high values of reproductive factor of *M. incognita*.

However, significant differences in the gall indices, egg and final nematode populations indicate different levels of susceptibility to *M. incognita* (Singh and Khurma, 2007). The variation in the susceptibility to *M. incognita* of the screened varieties maybe as a result of the genetic differences among the varieties and which also explains the variations in their final nematode populations. NHC 14, NHC 20 and NHC 25 were found to be highly susceptible to *M. incognita* as they supported multiplication of *M. incognita* which is shown in their gall indices, nematode population and reproductive factors. But despite the heavy infestation, their leaves and height were not greatly reduced.

This means these kenaf accessions can still be grown on *M. incognita*-infested soil, but will multiply nematodes on the field. The nematode build up in such field will in no doubt be injurious to successive crops, especially when they are susceptible to *Meloidogyne* species. The high levels of susceptibility as evident in gall indices shown by the kenaf accessions in this study is comparable to the values reported for okra (Akinlade and Adesiyan, 1982). This, no doubt corroborates the opinion of other workers that kenaf is another major host of root-knot nematodes (Dempsey, 1975; Lawrence and McLean, 1992; Adegbite *et al*., 2005). However, the good growth observed in some of the kenaf accessions despite their susceptible reactions might support the recent clamor by some workers for the inclusion of growth and yield parameters in the assignment of host resistance to root-knot nematodes (Atungwu *et al*., 2008). They opine that growth and yield factors which are of ultimate interest to farmers should be incorporated into the resistance rating scheme in order to make evaluation and selection of resistant cultivars more meaningful.

If this is done, resistant crop varieties to *M. incognita* and also with good yield will be selected for cultivation (Sasser *et al.* 1984; Atungwu *et al*., 2008). In resistant plants, nematodes fail to produce functional feeding sites in the host after invasion due to hypersensitive responses that leads to failure to produce mature females (Williamson and Kumar, 2006). Studies have shown that resistance can be broken by certain pathotypes of RKNs that are able to parasitize plants previously rated to be root-knot nematode resistant (Baicheva *et al.,* 2002; Abad *et al.,* 2003; Jacquet *et al.,* 2005). Such break in resistance is a major limiting factor in using plant resistance as a means for controlling RKN (Singh and Khurma, 2007). However, identification and use of RKN resistant and tolerant genotypes can still be a viable means of minimizing loss caused by RKN (Singh and Khurma, 2007).

Conclusion

This study showed that NHC-30 had the highest susceptibility to *M. incognita* among all kenaf accessions screened since it died at seven weeks after inoculation and thus not able to survive the infection. According to the reproductive factor, NHC-14, NHC-20 and NHC-25 were highly susceptible to *M. incognita* also. The best growth was seen in NHC-16, NHC-40 and NHC-400 and these can be cultivated for their leaves and other growth factors. NHC-28 was the outstanding accession considering level of resistance among other kenaf accessions screened since it showed the lowest gall index and reproductive factor. However, the NHC-28 was also susceptible to *M. incognita.*

Recommendations

NHC-28 is hereby recommended for field trials because of its excellent growth and slight susceptibility to *M. incognita* pending the discovery of resistant genotype(s). However, NHC-16, NHC-40 and NHC-400 may be cultivated if growth parameters such as leaves, plant height and stem diameter amongst others are desired attributes for kenaf production by the farmers. However, it is recommended that these kenaf accessions be rotated with resistant crops in order to avoid the build-up of *M. incognita* in the field which may be detrimental to cultivation of successive crops.

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