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SUSCEPTIBILITY AND TOLERANCE TO ROOT-KNOT NEMATODES (*MELOIDOGYNE INCOGNITA*) OF *TELFAIRIA OCCIDENTALIS* UNDER ORGANIC CONVERSION

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ABSTRACT

Three cultivars of *Telfairia occidentalis* were assessed for their resistance or otherwise to root-knot nematodes (*Meloidogyne* spp.) infection on the field. Seeds were extracted from the pods of the selected cultivars and planted on moist sawdust in nursery trays to raise seedlings for transplanting. The experiment was laid out in Randomized Complete Block Design and replicated thrice. Pre-plant soil samples were taken to estimate initial nematode populations. At seven days after transplanting, eggs of *Meloidogyne incognita* (Mi) were extracted from 60 day-old *Celosia argentea* and used for inoculation of the three cultivars of *T. occidentalis* at approximately 5000 eggs/stand. Plants were, thereafter, observed for vine length, vine girth and number of leaves from two weeks till 8 weeks after transplanting. Final soil nematode population and root gall indices were determined at 60-66 days after inoculation. Reproduction factor was determined and used along with the gall indices to rate the cultivars for resistance or susceptibility to Mi. Results showed that cultivars NHTo-020 and NHTo-030 were susceptible to root-knot nematode which implied that plants allowed nematode reproduction and also suffered yield loss while cultivar NHTo-010 was tolerant to nematode infestation meaning that nematode reproduction took place but the plant does not suffer yield loss. There was no significant (P>0.05) difference in the mean vine length and number of leaf irrespective of the inoculum while effect on the vine girth was significantly variable. The inoculated plants gave significantly better vegetative growth than the naturally infested plants which indicated genetic variability in the cultivars

Keywords: Phytonematodes, resistance, sustainable agriculture, vegetables, yield improvement.

INTRODUCTION

Organic agricultural production system is fast growing in Africa. However, there are concerns for pests and disease constraints in newly established organic farms due to restrictions from synthetic chemical applications. Plant-parasitic nematodes are among the major constraints in crop production system globally, including vegetables.

Fluted pumpkin (*Telfairia occidentalis* Hook. f.) is a tropical vine crop grown in West African countries for its leaves and seeds. Although, the crop is indigenous to Southern Nigeria (Akoroda 1990), it is now expansively cultivated or grown in home gardens in almost all the states in the country. The crop is a delicacy and commonly consumed by all tribes and large populations of foreigners in the country because of its nutritional, medicinal, economical, industrial and dietary importance to human The leaves, tender vines and seeds of *T. occidentalis* are rich sources of protein, oil, vitamins and minerals in addition to magnesium, calcium, sodium and phosphorus. It has been documented to have some medicinal values (Kayode and Kayode, 2011) for humans. It has been scientifically acknowledged that some phytochemicals in *T. occidentalis* can prevent or reduce oxidative damage of human body tissues hence can prevent cell death (Wei and Shiow 2001; Yang *et al*., 2002). Its inherent antioxidants help in reducing the free radicals generated from the oxidation processes that generate energy for the biological processes.

The fresh leaf extract serves as high value, cheap and fast remedy tonic for anaemia and convalescent persons in view of its high ferrous content which is about 700 ppm (Schippers, 2000). Harvested pumpkin can be eaten raw or processed by different methods and use in diverse ways depending on individual's culture (Aladetoyinbo, 2015). The mature vines constitute important fiber source in animal diet thereby serving as fodder for livestock.

In addition, fluted pumpkin is one of the appreciable cash generating crops in many parts of Nigeria (Akoroda, 1990) as it is considered as a pro-poor choice for money spinning since it can be pruned bi-weekly for regular marketing all-year-round. Income from the *Telfairia* fields may is dual in nature, from the leaves and pods which are usually very expensive during the commencement of the succeeding cropping season. Where residual soil moisture is sufficient, fluted pumpkin can grow for 2-3 years or more with adequate soil fertility maintenance.

In spite of the nutraceutical importance of fluted pumpkin to man, livestock and the ecosystem, very scanty literature exists on the status of the crop to plant-parasitic nematodes (farmers' hidden enemies) generally. Specifically, the cosmopolitan root-knot nematodes, *Meloidogyne* species which constitute up to 95-98% of all plant-parasitic nematodes globally, remain poorly documented in Nigeria and the West African sub-region particularly in *T. occidentalis*' fields. Of more concern is the likelihood of consequential threat of these nematodes in the emerging organic farmers' fields in Nigeria. Most of the time *Telfairia* is a succeeding crop in previously nematode-infested fields particularly other susceptible vegetables likes *Celosia argentea*, *Amaranthus* spp., *Solanum lycopersicum*, etcetera.

Meloidogyne spp. is an obligate parasite which is highly destructive to crops in nature. The infection of *Meloidoyne* spp. results in root gall disease, stunted growth and low productivity in plants (Pandey and Kaira, 2003), including vegetables (Atungwu *et al*., 2013). Fluted pumpkins are susceptible to root-knot caused by *Meloidogyne* spp. which as the primary factor can influence secondary infections by fungi, bacteria and viruses. The major contributions of modern plant breeding to fluted pumpkin production have been through the development of cultivars with improved disease resistance. As new cultivars are developed, there is need for their evaluation for resistance to root-knot nematode diseases. The objectives of this study were to assess the: 1) Host status of three cultivars of *Telfairia occidentalis* to *Meloidogyne* spp. under organic conversion, and 2) Effect of *Meloidogyne* spp. on the growth of the three cultivars of *Telfairia occidentalis* under organic conversion.

MATERIALS AND METHODS

The experiment was carried out on the field of Organic Agriculture Skill Development plot of the Federal University of Agriculture Abeokuta. Three cultivars of *T. occidentalis* (NHTo-010, NHTo-020 and NHTo-030) were obtained from National Horticultural Research Institute, Ibadan, Nigeria. Seeds were extracted from their pods separately and washed to remove the poisonous toxins before air drying overnight. Extracted seeds were planted individually in a moist saw dust in the nursery pots and watered daily until germination. Pre-plant nematode population was estimated using 18 soil samples that were collected randomly from each plot with the aid of the soil auger. Nematodes were extracted from the sub-samples of the soil using the Whitehead and Hemming (1965) nematode extraction techniques. Soil samples were taken to the laboratory where 250 g of each was weighed and placed in two plastic sieves sandwiched with a double-ply serviette tissue paper laid in a criss-cross method between the inner and outer sieves.

The set up was placed in a bowl into which water was added through the side of the sieve containing water and left undisturbed for 24 hours after which the sieve containing the soil was gently removed from the bowl. The nematode suspension in the bowl was poured into a 500 ml Nalgene bottle and water was added to fill to the brim of the bottle and it was left for 5 hours and then the excess content was siphoned out of the Nalgene bottle using a rubber tubing and the nematode suspension was poured into a sampling bottle and stored in the refrigerator. Nematodes present in each content were counted under the stereo microscope using the Doncaster (1962) counting dish.

After the initial nematode populations were determined, four week old seedlings were transplanted at 1 m x 1 m in the field in Randomized Completely Block Design comprising of the three cultivars, replicated three times. Total experimental size was 10 m x 5 m (50 m^2) which consisted of 18 experimental beds measuring 1 m x 1 m with a gang way of 0.5 m each. The seedlings were inoculated with approximately 5000 or zero *Meloidogyne* eggs one week after transplanting. Prior to inoculation, eggs of *Meloidogyne* spp. were extracted using the Chlorox method of Whitehead and Hemming (1965). The naturally infested plots with zero inoculum served as a control. In all the experimental plots, all the *Telfairia* plants were stalked with bamboo sticks measuring about 1.5 m high for the vines to climb easily.

Data were obtained on growth parameters which included vine length (cm), vine girth (cm) and number of leaves biweekly from two Weeks After Transplanting (WAT) until 8WAT. Soil samples were taken again at 60-66 Days After Transplanting (DAT) which coincided with 8WAT, to determine the population of *Meloidogyne* spp. per plot. Number of root galls were taken by carefully lifting up four previously tagged plants which were severed, washed under cool tap water, and observed in the laboratory. Thereafter, eggs were extracted and counted under the stereo microscope. Addition of the number of nematodes in the soil and eggs extracted from the plant roots form the final population of the target nematode, *Meloidogyne* spp. per plot. Final population and initial population was used to determine the reproduction factor of nematodes using Oostenbrink (1966) formula, (R= Pf/Pi). All the data obtained were subjected to Analysis of Variance. Afolami (2000) resistance rating scheme which integrated gall index, nematode reproduction factor and yield component was used for host designation of the selected *T. occidentalis* cultivars.

RESULTS

Table 1 showed the host status of three selected *T. occidentalis* resistance to root-knot nematode (*Meloidogyne incognita*). Gall index obtained were used along with the nematode reproduction factor and the yield difference between the inoculated and the nematode free plants. The all the three used for this study supported gall formation with the same Gall Index of 2.3 and had Reproduction Factor (Rf) over and above 1, ranging from 1.23 to 2,20, the yield of treated (inoculated) cultivars was only more in NHTo-010 (22.67 g) thus rated tolerant to *Meloidogyne* spp., compared with the decreasing yield in NHTo-020 (-1.23 g) and NHTo-030 (-12.67 g) rated as susceptible. The susceptible cultivars were observed to have not only supported high nematode populations but suffered significant decrease in economic yield. Contrary, cultivar NHTo-010 designated as tolerant was due to the fact that irrespective of high reproducibility of the nematode, it did not suffer yield loss.

Table 1: Resistance rating of three cultivars of *T. occidentalis* to Root-Knot nematode

Treatment	+Gall Index	++Reproduction Ractor	Inoculated (a)	Uninoculated Control (b)	*Difference (a-(:	Degree of Resistanc
NHTo-010	2.3	1.83	130.67	108.00	22.67	Tolerant
NHTo-020	2.3	2.20	121.67	133.00	-12.67	Susceptible
NHTo-030	2.3	1.23	152.00	159.67	-1.23	Susceptible

Key: + Gall Index rating from the scale of 0 – 5 where 0 = 0 gall, 1 = 1 – 2 galls, 2 = 3 – 10 gall, 3 = 11 – 30 galls, 4 = 31 – 100 galls and 5 = 100+ galls; ++Reproduction Factor (Rf) = Final Population (Pf)/Initial Population (Pi); *Yield of Inoculated Cultivar minus that of Uninoculated (Control)

Table 2 showed that there were no statistically significant ($p > 0.5$) differences in root and shoot weights as well as the root: shoot ration among the three selected *T. occidentalis* cultivars grown in *Meloidogyne* spp. naturally infested field under organic conversion, irrespective of the batch that were inoculated with 5,000 eggs.

The effects of root-knot nematodes species on the vine lengths of three *T. occidentalis* on a field being converted to organic agricultural production system are shown on Table 3. Between the second and sixth weeks after inoculation, there was no statistically variable ($p > 0.05$) performance of the three cultivars whether treated or not treated (control). However, at 60 days after inoculation, representing 8WAI, significant ($p < 0.05$) differences were recorded in the vine lengths of the three cultivars inoculated or uninoculated. The longest ($p < 0.05$) vine was 1. 56 m in NHTo-020 that did not receive addition (inoculation) of the root-knot nematode species which was not in any case longer than the 1.15 m vine length of its inoculated counterpart , nor the 1.14 m, 1.22 m, 86 cm lengths of vines in NHTo-010 (uninoculated), NHTo-030 (uninoculated) and NHTo-010 (inoculated) *T. occidentalis* cultivars. The vine length of uninoculated NHTo-020 differed significantly from those of NHTo-010 (inoculated and uninoculated) and the inoculated NHTo-030 cultivars. The responses the vine girths of three *T. occidentalis* to root-knot nematodes species in a field undergoing organic farming conversion are presented on Table 4.

Table 5 showed the effects of *Meloidogyne* spp. on the number of leaves of three *T. occidentalis* evaluated on a field being converted to organic farming system. From 2WAI to 4WAI, no statistical variability ($p > 0.05$) in number of leaves was observed among the three cultivars and the treatments. At 6WAI and 8WAI, statistically significant ($p < 0.05$) differences were found in the number of leaves, with the largest ($p < 0.05$) being 80 in NHTo-030 (uninoculated) which differed from 60 in NHTo-020 (inoculated) cultivar. Uninoculated NHTo-030 cultivar had the largest ($p < 0.05$) number of leaves (114) at 8WAI which differed from the 82 in in NHTo-020 (inoculated) cultivar but did not differ ($p > 0.05$) statistically from all other treatments evaluated.

Table 2: Responses of root and shoot weights to root-knot nematode-infected *Telfairia occidentalis*
Means followed by the same letter in the same column are not significantly different according to Duncan Multiple Range Test

Treatment	Root Weight (g)	Shoot Weight (g)	Root Weight (g)	Root: Shoot Ratio
NHTo-010 Inoculated	141.00a	16.00a	130.67a	1:8a
NHTo-010 Uninoculated	127.00a	18.67a	108.00a	1:6a
NHTo-020 Inoculated	143.00a	21.67a	121.67a	1:5a
NHTo-020 Uninoculated	143.00a	22.00a	133.00a	1:6a
NHTo-030 Inoculated	177.67a	23.00a	152.00a	1:6a
NHTo-030 Uninoculated	179.67a	20.00a	159.67a	1:7a

Table 3: Effect of root-knot nematodes on the mean vine length (cm) on *Telfairia occidentalis*

Treatment+	Vine Length 2WAI++	Vine Length 4WAI	Vine Length 6WAI	Vine Length 8WAI
NHTo-010 inoculated	16.00a	57.33a	68.67a	86.33b
NHTo-010 uninoculated	16.00a	59.33a	66.33a	114.33b
NHTo-020 inoculated	17.33a	55.00a	71.00a	120.33ab
NHTo-020 uninoculated	14.67a	53.67a	92.33a	156.33a
NHTo-030 inoculated	14.33a	49.00a	63.33a	115.00b
NHTo-030 uninoculated	15.00a	51.33a	64.00a	121.67ab

+ Means followed by the same letters in the same column are not significantly different. ++WAI: Week after inoculation

Table 4: Stem girth of root-knot nematode-infected *Telfairia occidentalis* cultivars under organic farming conversion

+Means followed by the same letters in the same column are not significantly different. ++WAI: Week after inoculation

Treatment+	Vine Girth (cm) 2WAI ++	Vine Girth (cm) 2WAI	Vine Girth (cm) 2WAI	Vine Girth (cm) 2WAI
NHTo-010 inoculated	19.67a	24.67a	26.33a	27.33a
NHTo-010 uninoculated	22.33a	26.00a	27.67a	29.67a
NHTo-020 inoculated	21.33a	25.67a	25.67a	28.00a
NHTo-020 uninoculated	20.67a	25.33a	25.00a	28.00a
NHTo-030 inoculated	19.33a	37.00a	23.33a	25.67a
NHTo-030 uninoculated	21.00a	23.00a	25.67a	30.33a

Table 5: Effects of root-knot nematodes species on the number of leaves of three *Telfairia occidentalis*

Treatment	NL2WAI	NL4WAI	NL6WAI	NL8WAI
NHTo-010 inoculated	19.00a	38.00a	76.67ab	97.67ab
NHTo-010 uninoculated	20.67a	41.67a	78.00ab	104.00a
NHTo-020 inoculated	20.00a	37.33a	60.33b	82.33b
NHTo-020 uninoculated	17.67a	39.67a	73.67ab	96.33ab
NHTo-030 inoculated	17.67a	40.67a	73.00ab	102.00a
NHTo-030 uninoculated	19.33a	45.00a	80.00a	114.00a

+Means followed by the same letters in the same column are not significantly different.
++WAI: Week after inoculation.

DISCUSSION

Plant-parasitic nematodes (PPN) generally associate with cultivated and wild or forest plants (Atungwu and Soneye, 2016) across the world. Root-knot nematodes (*Meloidogyne* species) represents 95 – 98 % of all global PPN that can cause economic consequences to crop and agricultural production. Plant-*Meloidogyne* interactions can be evaluated from the damage to the roots and its resultant effect above-ground such as number of leaves, stem girth, plant height, number of branches and other agronomic parameters. The nematodes have the capacity to cause gall formation which is a disease condition that is below-ground. The galls are used to determine the host status of the invading plants. The number of galls produced by the infected plants is an indication of the extent of the root damage by this species (Afolami, 2000) of herbivorous nematodes. The systematic way of rating crops for root-knot nematodes resistance currently assures that the suggestion by Afolami (2000) to improve the traditional Taylor and Sasser (1978) gall index scale and Sasser *et al*. (1984) Host suitability designation scheme, was an immense improvement that carefully determine the d scheme that integrated the modification that included rating on the effects on the economic (yield) plant part as a more meaningful host status determination.

Traditional, conventional and organic crop production systems are all affected by one or more herbivorous nematodes. In newly organic system or those undergoing conversion, nematode pest or disease management is quite a herculean task as nematicides are usually excluded from the system. In addition, the host status of the selected crop cultivars will determine its vulnerability or otherwise in the management of the pest. Resistant cultivars and varieties are prime choices and a recommendation for organic crop production system. There appeared to be scarce information on availability of enough certified organic seeds for the emerging African farmers in the sector.

It was in view of this that the three commonly grown *T. occidentalis* cultivars were evaluated for their reactions to *Meloidogyne* spp. to verify their status to the nematodes. The study clearly observed variability in the reactions of the three cultivars with none being resistant. Up to 75% were found to be susceptible and 25% tolerant. The tolerant cultivar was so designated because of the fact that its economic yield (leaves in this case) was not affected by the nematodes even though they allowed root damage and supported high population of the nematodes. This observation on the larger yield (leaves) was in agreement with the earlier findings of Oyedunmade and Fatoki (1995) who reported in addition to the increased number of leaves, higher plant height and reduced root galls in okra sown in *Meloidogyne incognita*-infested soils.

The nature of resistance of root-knot nematode was variable in the three cultivars of *Telfairia occidentalis* both in the inoculated and in the uninoculated plant. The reduced plant growth could be due to root destruction by root-knot nematode and utilization of nutrients and related resources by the galled roots to the detriments of the plants. *Meloidogyne* infection results in nutrient or metabolic sink as the manufactured food is re-directed to the roots to meet the parasitic needs of the nematodes which was reported by Abbas *et al*. (2009).

Nematode feeding results in the increase of root weight because of the galls which have negative effect on shoot weight and causes reduction in foliage at increased inoculum (Khan, 2009). The inclusion of *T. occidentalis* leaf yield is the actual target of the farmers, as additional parameters for measuring plant parameters as recommended by Afolami (2000), which ensured a more meaningful designation of host status of the *Telfairia* cultivars used in the study. This study therefore, showed from the yield loss assessment of the cultivars that tolerant cultivars has superior yield compared to the susceptible cultivars whose yield loss was affected by root-knot nematode infection even though both tolerant and susceptible cultivars had similar gall indices and nematode reproduction factor.

CONCLUSION

This study concluded that two cultivars of *Telfairia occidentalis*, NHTo-020, NHTo-020 and NHTo-030, were susceptible to root-knot nematodes (*Meloidogyne* spp.), and one (NHTo-010) out of the three was tolerant to the nematode.

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