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# RESPONSE OF SELECTED TREE SEEDLINGS TO ROOT-KNOT NEMATODE (*Meloidogyne incognita*)

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**ABSTRACT**

Root-knot nematodes have been implicated in poor growth and death of trees in the nursery and newly established forest plantations. Cultivating resistant tree species is one of the viable methods in the management of nematode pests once they are identified. A pot experiment laid out in completely randomized design was conducted to screen fifteen selected tree seedlings for resistance to *Meloidogyne incognita.* Each tree seedling was replicated eight times and was grown on a steam-sterilized soil. The seedlings were inoculated each with 7,000 eggs of *Meloidogyne incognita* at four weeks after planting. Data were collected on plant height and stem girth at 12 weeks after inoculation. At termination, data were also collected on gall index (GI), final nematode population (Pf) and reproductive factor (RF). Data were analyzed using analysis of variance. Resistance or susceptibility status was assigned with Canto-Saenz host designation scheme. There was variability in the growth of the tree seedlings. *Simphonia africana* had the highest GI, Pf and RF of 3.3, 54,500 and 22.07, respectively and these values were significantly higher than values from other seedlings. Six tree seedlings; *Simphonia africana, Theobroma cacao, Gmelina arborea, Piptadeniastrum africana, Chrysophyluum albidium* and *Parkia biglobosa* were found to be susceptible to *M. incognita*, while six (*Tetrapleura tetraptera, Anacardium occidentale, Annonas muricata, Dinium guinensis* and *Vitex doniana*) were found to be resistant and three (*Treculia africana*, *Mangifera indica* and *Dacryodes edulis*) were tolerant. Resistant tree seedlings should be planted in *M. incognita*-infested plantation and susceptible species should be treated even in the nursery.

***Keywords:*** *Meloidogyne incognita*, Nematode population, Reproductive factor, Resistance, Tree seedlings.

## INTRODUCTION

The outstanding values of natural forest and forest plantation trees to the ecosystem such as amelioration of climate change, provision of clean air, prevention of water pollution, prevention of soil erosion, providing food especially for wildlife and birds amongst others place necessity on the management of constraints to their survival. There have been several reports of death of many tree seedlings at the nursery stage and young tree seedlings at newly established forest plantations owing to several factors (Barber, 2004; Labode, 2014). Some of these factors are edaphic, climatic and biotic in nature. In spite of the benefits of trees to mankind, they suffer notable damages from pests and pathogens. Significant among these biotic constraints to forest establishment and growth of trees are insects such as *Symphyta, Acraea, Andromache* and pathogens such as fungi (*Armillaria* root disease on tree roots), viruses, bacteria and plant-parasitic nematodes (Lownsbery and Lownsbery, 1985; Khan, 2012).

Nematode pests are one of the major pathogens causing diseases in forest plantations thereby threatening their survival and productivity. Plant-parasitic nematodes have been implicated in the death of many trees at the seedling stage and newly established forest plantations (Khan, 2012; Sigariova and Karpliyk, 2015). Most tree seedlings in the nursery do not successfully become established in the forests due to death caused by pests and also, most forest plantations have been wiped out due to diseases by pathogens such as plant-parasitic nematodes (Barber, 2004; Pimentel *et al*., 2005). However, nematode pests of forest trees either in plantation or in the nursery are less reported with little or no knowledge of them especially in developing countries of the world (Khan, 2012). This may be due to difficulty in diagnosis of nematode problems and evaluating their effects on well established trees with characteristic little or no symptoms.

*Meloidogyne* species, *Bursaphelenchus* spp. and *Xiphinema americanum* have been found to be one of the common and damaging nematode pests on trees (Lownsbery and Lownsbery, 1985; Khan, 2012). Sigariova and Karpliyk (2015) studied composition of plant-parasitic nematodes in the flowering and tree seedlings cultivated at the greenhouse facilities. In the study, different species from the genera *Meloidogyne*, *Rotylenchus, Tylenchorhynchus*, *Helicotylenchus*, *Paratylenchus* and *Heterodera* were discovered. Infected plants by *Meloidogyne* species usually develop large root galls causing root-knot disease. McSorley and Parrado (1986) were able to relate amount of root galling to the growth in terms of plant height, the number of leaves and stem girth of tree seedlings owing to root-knot disease by *Meloidogyne incognita*. The disease affects adversely both leaves and roots of trees. Plant height, stem girth and number of leaves have been reported to be reduced significantly with severe stunted, chlorotic and wilted seedlings at high population of *Meloidogyne incognita* (Barillas –Argueta,

1949; Cram and Fraedrich, 2012).

Forest plantation trees and plants are susceptible to root-knot nematodes, *Meloidogyne* species (Adeniji, 1970; Saucet *et al*., 2016; Khan, 2020). Some researchers reported increased mortality of trees in plantation with high population of *M. incognita*. Management of root-knot nematodes adverse effects on trees has not been explored significantly yet using resistance in tree species. Genetic tactics to combat adverse effects of pests and pathogens on forest trees are being strongly advocated (Manion, 1991; McLean, 1998). This involves selection of highly disease resistant tree genotypes over the susceptible ones. The genetic variation in disease resistance can then be explored in managing any pests and pathogens. Planting of resistant tree species have been reported in the management of some pathogens such as fungi and bacteria in Brazil and South Africa, but little or no report exist on plant-parasitic nematodes (Barber, 2004). Thus, resistant tree plants and the basis of their resistance need to be identified to manage plant-parasitic nematodes (Barber, 2004; Starr *et al*., 2010). This study was carried out to evaluate the resistance status of selected fifteen tree seedlings to *Meloidogyne incognita.*

## MATERIALS AND METHODS

### Experimental Location

This study was conducted at the Screenhouse of the Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt, Port Harcourt, Rivers State. The screen house lies on latitude 04.538$^{o}$ 33''N and longitude 0.06$^{0}$54.38 0''E in Southern Nigeria.

### Source of Tree Seedlings

The fifteen tree seedlings used for this study were obtained from the Arboretum, Department of Forestry and Wildlife, University of Port Harcourt. The seedlings were *Piptadeniastrum africana, Azadirachta indica, Chrysophyllum albidum, Parkia biglobosa, Mangifera indica, Tetrapleura tetraptera, Annonas muricata, Vitex doniana, Gmelina arborea, Dacryodes edulis, Dinium guineensis, Anacardium occidentale, Treculia africana, Theobroma cacao,* and *Simphonia africana.*

### Preparation of Experimental Pots and Propagation of Test Plants

Eight-litre black polythene bags served as pots and they were perforated to allow for even drainage. These pots were filled with 7 kg of steam-sterilized top soil so as to facilitate the inoculation with 7,000 eggs of *M. incognita*. This study was set up in completely randomized design (CRD). Fifteen tree seedlings were each planted in polythene bags containing 7 kg sterilized soil. Each tree seedling was replicated eight times and randomly arranged in the screenhouse of the Department of Crop and Soil Science, University of Port Harcourt. The trial was repeated with no modifications.

### Test of Resistance of Tree Seedlings against *Meloidogyne incognita*

Already established four weeks old tree seedlings had four holes dug at a depth of 3 cm round the roots of each tree seedling. The inoculation was carried out with 2 ml of *M. incognita* egg suspension containing 7,000 eggs drawn with a 2 ml hypodermic syringe subsequently released into the holes made and later covered with soil.

### Collection of Data

The performance of the fifteen tree seedlings before and after inoculation with *M. incognita* was evaluated by taking data on plant height (cm), number of leaves visually counted, and stem girth (mm) at weekly interval until the experiment was terminated. At the end of the experiment, each tree seedling was partitioned into shoot and roots. The fresh shoot weight (g) was taken using an electronic balance. The root system of each tree seedling was carefully dug out. Galls on the roots were rated by counting the number of galls using the scale 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. The entire root system of each plant was 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 chopped roots were placed in a conical flask containing 0.5% sodium hypochlorite solution of 100 ml and shaken vigorously for 4 minutes. The content was poured into stack sieves of 200 mesh nested over 500 mesh. 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. The egg suspension was thoroughly mixed with a magnetic stirrer and 2 ml aliquot of the egg suspension drawn with a hypodermic syringe was released into a Doncaster dish and counted with the aid of a tally counter under a compound microscope.

The second-stage juveniles (J2) in the soil per pot was extracted using the modified Baermann method (Whitehead and Hemming, 1965; Coyne *et al*. (2007). The infested soil was thoroughly mixed together to attain homogeneity and sieved to remove stones and debris. 200 cm$^3$ of sieved soil sample was collected with the use of a beaker and then placed on a facial tissue in a plastic sieve and water added to the extraction plates by the sides. The set-up was allowed to stay for 48 hours. The suspension in the extraction plate was poured into a beaker, allowed to settle. Second-stage juveniles of *M. incognita* were extracted using modified Baermann technique (Whitehead and Hemming, 1965; Coyne *et al*., 2007). The infested soil was thoroughly mixed together to attain homogeneity and sieved to remove stones and debris. 200 cm$^3$ of sieved soil sample was collected with the use of a beaker and then placed on a facial tissue in a plastic sieve and water added to the extraction plates by the sides. The suspension in the extraction plate was poured into a beaker, allowed to settle. The suspension was gently decanted and the juvenile population was determined in similar manner to egg population. The total number of second-stage juveniles in the soil was extrapolated from the number of second-stage juvenile in 200 cm$^3$ soil. The number of nematodes in the soil was added to the number of eggs extracted from the root to obtain the final nematode population (Pf). The host efficiency was determined by the reproduction factor (RF) = Pf/Pi, which was calculated; where Pf (final nematode population) and Pi = 7,000 eggs, the initial nematode population density. A reproduction factor of greater than 1 indicates an increase in nematode reproduction where an RF factor of less than1 implies no increase in reproduction. The final assessment of resistance of these seedlings was based on Canto-Saenz host designation scheme (Sasser *et al*., 1984). Plants with GI (Gall index) greater than 2 are defined as either susceptible (RF greater than1) or hyper susceptible (RF less than 1); plants with

Gall index less than 2 are classified either resistant (RF less than 1) or tolerant (RF greater than 1).

**Data Analysis**

Nematode counts were prior transformed with $\log_{10}(x+1)$. Data collected were analyzed using analysis of variance and means separated with Fisher's least significant difference (LSD) test at 5% level of probability using the Statistical Analysis System (SAS, 2009).

**RESULTS**

Table 1 **s**hows the effects of *Meloidogyne incognita* on growth parameters of fifteen tree seedlings. At termination of experiment, *Vitex doniana* had the highest mean number of leaves which was not significantly higher than mean number of leaves in *Annonas muricata*, *Gmelina arborea* and *Dinium guinensis*. The least mean number of leaves was recorded in *Parkia biglobosa* and *Chrysophyllum albidum*. *Gmelina arborea* had the highest mean plant height which was significantly higher than mean plant heights of other tree seedlings. *Chrysophyllum albidum* had the least mean plant height among all the tree seedlings screened against *M. incognita* (Table 1). *Gmelina arborea* had the highest mean stem girth that was significantly higher than stem girths of other tree seedlings. *Chrysophyllum albidum* had the least stem girth among the fifteen tree seedlings.

**Table 1: Effects of *Meloidogyne incognita* on fifteen tree seedlings in the nursery**

| Tree species | Number of leaves | Plant height (cm) | Stem girth (mm) |
|---|---|---|---|
| *Gmelina arborea* | 22.500 | 93.6 | 5.08 |
| *Vitex doniana* | 24.75 | 35.20 | 2.78 |
| *Annonas muricata* | 22.50 | 39.25 | 2.65 |
| *Dialium guinensis* | 22.25 | 38.0 | 2.40 |
| *Tetrapleura tetraptera* | 14.25 | 45.85 | 3.05 |
| *Simphonia africana* | 14.50 | 37.28 | 2.55 |
| *Anacardium occidentale* | 18.00 | 76.65 | 3.55 |
| *Theobroma cacao* | 12.50 | 55.0 | 3.43 |
| *Dacryodes edulis* | 16.00 | 37.18 | 3.03 |
| *Azadirachta indica* | 10.00 | 35.90 | 2.75 |
| *Parkia biglobosa* | 6.75 | 40.90 | 2.25 |
| *Piptadeniastrum africana* | 9.50 | 35.52 | 2.80 |
| *Mangifera indica* | 12.25 | 45.73 | 2.73 |
| *Treculia africana* | 9.25 | 28.53 | 2.55 |
| *Chrysophyllum albidum* | 6.75 | 18.10 | 1.52 |
| LSD ($P \leq 0.05$) | 5.83 | 10.51 | 0.45 |

Table 2 shows the effects of *Meloidogyne incognita* on mean gall index, egg population, second stage juvenile (J2), final nematode population (PF) and reproductive factor (RF) of 15 tree seedlings**.** *Simphonia africana* were heavily galled having the highest gall index of 3.25 which was not significantly higher than gall indices of *Theobroma* cacao and *Gmelina arborea*. Also, *Simphonia africana* had the highest *M. incognita* egg population of 138,500 and this was significantly higher than egg population of other tree seedlings. The highest nematode population of 154, 500 was recorded for *Simphonia africana*. *Simphonia africana* highly supported the reproduction of nematode having the highest reproductive factor of 22.07 which was significantly higher than reproductive factor from other tree seedlings. *Vitex doniana* had the lowest reproductive factor of 0.24 among the tree seedlings.

Table 3 showed the final assessment of resistance to 15 tree seedlings based on Canto-Saenz host designation scheme. When the root gall index value is greater than or equal to 2 and also the R-factor is greater than or equal to 1, it showed that the plant is highly susceptible to *Meloidogyne incognita.* Therefore, based on Canto-Saenz resistance ratings of the 15 tree seedlings, six were susceptible to *Meloidogyne incognita*; six were resistant since their root gall indices value is less than or equal to 2 and the R-factor value is less than or equal to 1. Three tree seedlings were tolerant to *Meloidogyne incognita* because the root gall index value is less than or equal to 2 and the R-factor is greater than or equal to 1.

**Table 2: Effects of *Meloidogyne incognita* on mean gall index, egg population, second stage juvenile population, final nematode population and reproductive factor of 15 tree seedlings**

| Tree species | Gall index | Egg population | Second-stage juvenile population | Final nematode population | Reproductive factor |
|---|---|---|---|---|---|
| *Simphonia africana* | 3.3 | 138500 | 16000 | 154500 | 22.07 |
| *Theobroma cacao* | 3.0 | 65000 | 62500 | 71250 | 10.17 |
| *Gmelina arborea* | 2.8 | 115000 | 6500 | 121500 | 17.36 |
| *Piptadeniastrum africana* | 2.5 | 40000 | 3400 | 43400 | 6.20 |
| *Chrysophyllum albidium* | 2.5 | 47500 | 5000 | 52500 | 7.50 |
| *Parkia biglobosa* | 2.0 | 37500 | 2800 | 40300 | 5.76 |
| *Treculia africana* | 1.3 | 9000 | 700 | 9700 | 1.39 |
| *Tetrapleura tetraptera* | 1.3 | 3250 | 800 | 4050 | 0.58 |
| *Anacardium occidentale* | 1.3 | 5500 | 800 | 6300 | 0.90 |
| *Annonas muricata* | 1.0 | 1000 | 800 | 1800 | 0.26 |
| *Azadirachta indica* | 1.0 | 3000 | 1300 | 4300 | 0.61 |
| *Mangifera indica* | 1.0 | 6600 | 2700 | 9300 | 1.33 |
| *Dacryodes edulis* | 1.0 | 12000 | 1100 | 13100 | 1.87 |
| *Dinium guinensis* | 1.0 | 1700 | 550 | 2250 | 0.32 |
| *Vitex doniana* | 1.0 | 1300 | 400 | 1700 | 0.24 |
| LSD(P≤0.05) | 0.51 | 21747 | 1447.7 | 22672 | 3.24 |

**Table 3 Canto-Saenz host designation of fifteen tree species for resistance to *Meloidogyne incognita***

| Tree species | Gall index | Reproductive factor | Designation |
|---|---|---|---|
| *Simphonia africana* | 3.25 | 22.07 | Susceptible |
| *Theobroma cacao* | 3.0 | 10.17 | Susceptible |
| *Gmelina arborea* | 2.8 | 17.36 | Susceptible |
| *Piptadeniastrum africana* | 2.5 | 6.20 | Susceptible |
| *Chrysophyllum albidium* | 2.5 | 7.50 | Susceptible |
| *Parkia biglobosa* | 2.0 | 5.76 | Susceptible |
| *Treculia africana* | 1.3 | 1.39 | Tolerant |
| *Tetrapleura tetraptera* | 1.3 | 0.58 | Resistant |
| *Anacardium occidentale* | 1.3 | 0.90 | Resistant |
| *Annonas muricate* | 1.0 | 0.26 | Resistant |
| *Azadirachta indica* | 1.0 | 0.61 | Resistant |
| *Mangifera indica* | 1.0 | 1.33 | Tolerant |
| *Dacryodes edulis* | 1.0 | 1.87 | Tolerant |
| *Dinium guinensis* | 1.0 | 0.32 | Resistant |
| *Vitex doniana* | 1.0 | 0.24 | Resistant |
| LSD(P≤0.05) | 0.51 | 3.24 | |

## DISCUSSION

The penetration of a plant by *M. incognita* depends on the ability of *M. incognita* juveniles to gain entry into the roots of the plants and cause the formation of giant cells and subsequently development of knots (galls) on the roots (Chen *et al*., 2004). The presence of galls of diverse magnitudes on roots of all the fifteen tree seedlings indicates penetration by *M. incognita* and differences in severity of galling (damage) (Grauke and Starr, 2014). This damage by *M. incognita* observed in this study based on gall indices ranged from moderate to severe nematode damage. However, significant differences in the gall indices and egg population indicate different levels of susceptibility, resistance and tolerance to *M. incognita* (Singh and Khurma, 2007). *Meloidogyne incognita* has been implicated as one of the sources of stress to forest trees contributing to decline in productivity on penetration (Lownsbery and Lownsbery, 1985; Saucet *et al*., 2016). *Simphonia africana, Theobroma cacao, Gmelina arborea, Piptadeniastrum africana, Chrysophyllum albidum* and *Parkia biglobosa* were found to be highly susceptible to *M. incognita* as they supported multiplication of *M. incognita* as shown in their high gall indices. Other workers have reported the susceptibility of some tree species to root-knot nematodes where they contributed to damages and even death (Nyzcepir and Wood, 2012; Grauke and Starr, 2014; Saucet *et al*., 2016). The tolerant species, despite the heavy infestation, their leaves and height were not greatly reduced. This means

they can still be grown, but will multiply nematodes in the nursey and plantation. There is still need to manage nematode pests when tolerant species are cultivated using appropriate measures so as not to build up population of *M. incognita* in the forest plantation.

Some of the tree seedlings (*Tetrapleura tetraptera, Anacardium occidentale, Annonas muricata, Azadirachta indica, Dinium guinensis, Vitex doniana*) screened in this study were resistant to *M. incognita* and as such did not support reproduction and subsequently damage. In resistant plants, nematodes fail to produce functional feeding sites in the host after invasion due to hypersensitive responses that leads to failure to develop as reproducing females (Williamson and Kumar, 2006). Resistance to root-knot nematodes has been identified in Peach, Coffee and Almond trees of the *Prunnus* species. In these species and their accessions, single dominant *R gene, Ma* have been reported to confer high degree of resistance to *M. incognita, M. arenaria* and *M. javanica* (Lecouls *et al*., 1997; Rubio-Cabetas *et al*., 1998; Saucet *et al*., 2016). Such identified genes can be incorporated into tree breeding programmes for resistance to nematodes and other pests. Screening of more tree seedlings for resistance to nematode pests and other pathogens will lead to discovery of more resistant genes. Some resistance-breaking pathotypes of root-knot nematodes have been reported in the breakdown of some root-knot nematodes resistant plants (Baicheva *et al.,* 2002*;* Jacquet *et al*., 2005). However, identification and use of root-knot nematodes resistant and tolerant tree seedlings still offers viable means of minimizing losses caused by these nematodes in forest plantations over synthetic nematicides (Singh and Khurma, 2007). The introduction of already identified resistant genes into susceptible tree species during breeding has been reported promising in the management of *Meloidogyne* species and this should be encouraged to ensure improve tree growth (Saucet *et al*., 2016).

**CONCLUSION**

Six tree seedlings; *Tetrapleura tetraptera, Anacardium occidentale, Annonas muricata, Azadirachta indica, Dinium guinensis,* and *Vitex doniana* were resistant to *M. incognita*. After further screening, the genes conferring resistance on these so identified tree seedlings should be identified. Also, management approaches should be developed for the susceptible tree species.

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