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PATHOGENICITY OF *MELOIDOGYNE INCOGNITA* ON *SESAMUM INDICUM* AND THE EFFICACY OF YIELD-BASED SCHEME IN RESISTANCE DESIGNATION

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Abstract: Two screenhouse experiments were conducted in 2004 and 2005 rainy season to investigate the reaction of three selected *Sesamum indicum* cultivars against three population densities of a root knot nematode, *Meloidogyne incognita*. Seedlings of *S. indicum* were raised in pots arranged in completely randomised design and inoculated with 0, 5000, and 10000 eggs of *M. incognita*, replicated six times. Root knot disease was evaluated at mid-season and harvest. A new method for evaluating and reporting resistance to *Meloidogyne* spp. that divides the screening procedure into two phases in the same experiment was adapted. The first phase investigated the host response through the traditional standard method that utilises only gall and nematode reproduction indices, while the second considered the effect of root knot disease on grain production of the crop. There was consistency in host designation of E8 and NICRIBEN-01M (syn: 530-1-6) which were classified under the traditional and improved rating schemes as tolerant and resistant, respectively. However, *S. indicum* breeding line Pbtil (No. 1) which was considered susceptible under the old system was found to be tolerant using the integrated and improved system. Root galls incited by the nematode degenerated significantly from mid-season to harvest time. Utilising yield as additional parameter for assessing resistance to root knot nematode provides a complete picture of *Sesamum–Meloidogyne* interaction, and therefore a more meaningful system for determining host response.

Key words: Galls, grain yield, resistance, root knot nematodes, sesame.

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INTRODUCTION

Crop improvement objectives usually focus on increasing the quantity and quality of crop produce. The overall effect of *Meloidogyne* spp. which are cosmopolitan pests and occur in association with a wide range of crop plants in the temperate, tropical, and sub-tropical regions of the world, is reduction in quality and quantity of crop yield (Adesiyan *et al.* 1990). Three species namely, *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* have been found in Nigeria. *M. incognita* is the predominant species occuring in Southwestern Nigeria, where the nematodes negatively impacted on growth and grain yields of several commonly cultivated crops including *Abelmoschus* spp. (Afolami and Adigbo 1999), *Glycine max* (Afolami 2000), *Oryza sativa* (Afolami 2001; Afolami and Orisajo 2003), *Vigna unguiculata* (Odeyemi 2004), among others.

Effective nematode control and management options are numerous. They vary from nematicide application to non-chemical alternatives. The use of synthetic nematicides for management of outbreaks of root knot nematodes have become increasing costly (Kratochvil *et al.* 2004) for resource-poor farmers who produce the bulk of food in sub-Saharan Africa. Crop rotation involving the use of non-host or resistant varieties (Kratochvil *et al.* 2004) is the oldest proven method of nematode control and management (Egunjobi 1992). Adesiyan *et al.* (1990) advocated careful screening and selection of resistant varieties to demonstrate the effectiveness and significance of nematode population management through the use of resistant crop plants. *Sesamum indicum*, a commercial oilseed in Nigeria, is well known elsewhere to have dual advantage of high nutritional importance (Oplinger *et al.* 1990; Dudley *et al.* 2000) and *Meloidogyne*-resistance potentials (Rodriguez-Kabana *et al.* 1988), thus making Araya and Caswell-Chen (1994) to recommend *S. indicum* as an economic crop rotation component that would inhibit or restrict penetration and reproduction of *M. incognita* thereby reducing their populations in agricultural soils.

However, Atungwu *et al.* (2003, 2005) attempted screening 14 accessions of *S. indicum* for resistance to *M. incognita* and reported 86% resistance of sesame to the nematode at a standard inoculum level of 5 000 eggs per plant. However, their screening exercise was considered preliminary since they utilised Sasser *et al.* (1984) host suitability scheme for resistance to *M. incognita.* Although Sasser and co-workers' resistance rating system is well known and has continued to be adopted globally, Afolami (2000) emphasised that any rating scheme that neglects the important aspect of how the nematode damage affects economic yield which is the target of the farmer, is subjective. Confirmatory results (Afolami 2001; Afolami and Orisajo 2003; Odeyemi 2004) on the superiority in the efficacy of the yield-based rating scheme over gall index and nematode reproduction indices alone calls for adoption of the new scheme as a modification of the Sasser *et al.*'s (1984) scheme for rating crops for resistance to root-knot nematodes. Therefore, the objectives of this study were to:

1. confirm. the host status of *S. indicum* to *M. incognita*
2. investigate the pathogenicity of *M. incognita* to the crop at varying inoculum levels.

MATERIALS AND METHODS

Studies were conducted at the screenhouse of the Department of Crop Protection at the University of Agriculture, Abeokuta (UNAAB), South-Western Nigeria, to investigate the sensitivity or otherwise of three *S. indicum* genotypes to different *M. incognita* poulation densities and their interactions. The genotypes which were obtained from the National Cereals Research Institute, Badeggi, Nigeria, include E8, Pbtil (No. 1) and NICRIBEN-01M, with known resistance, tolerance and susceptibility reactions, respectively to a standard *M. incognita* inoculum. NICRIBEN-01M variety is widely cultivated by farmers throughout Nigeria. Sandy loam top soil (87.0% sand, 9.7% silt, 3.3% clay, pH 5.84 at 29°C) used for this investigations was sourced from UNAAB teaching and research farms (7°.15^{1}N and 3°. 25^{1}E), and was heat-sterilized for 90 minutes at 65°C with the aid of electric sterilizer. Sterilized soil was rested for six weeks in jute sacs to restore soil stability before planting. Thereafter, 4 kg of the homogenised soil was uniformly distributed into six litre plastic buckets used as pots, and arranged in completely randomised design with three replications each for mid-season and harvest data collection. Six seeds each of the *S. indicum* were sown per pot but selectively thinned to one seedling per pot six days after emergence.

Seven days after emergence, the soil with seedlings of *S. indicum* was inoculated with 0, 5 000 or 10 000 pure culture of *M. incognita* eggs obtained earlier by propagation of nematodes on susceptible *Celosia argentea* variety, TVL8 obtained from the National Horticultural Research Institute, Ibadan, South-Western Nigeria. Eggs were obtained from 60 day-old infected roots of TVL8 *C. argentea*, using Hussey and Barker (1973) extraction method by shaking segments of a clean roots for five minutes in 0.52% sodium hypochlorite solution in 250 ml conical flask. The resulting egg suspension was quickly poured into a 200-mesh sieve nested upon a 500-mesh sieve. Eggs caught in the 500-mesh sieve were then rinsed under gentle stream of cool tap water for four minutes and enumerated in a Doncaster (1962) counting dish under stereo microscope.

Seedlings were maintained for optimal growth by wetting as necessary every morning. Plants were observed for appearance of symptoms. At 60 days after inoculation, three replicates each were carefully lifted from the pots to avoid any damage to the roots. Galls on each root system were counted after which eggs were uniformly freed from the roots as previously described, but with 1% NaOCl for maximum recovery. Nematodes were extracted from the 250 g sub-samples of respective soil using modification of WhiteHead and Hemming (1965) tray method. Soil was placed in two 19.5 cm inside diameter plastic seives sandwitched with double-ply extractor tissue paper and placed in 26 cm inside diameter plastic bowl containing 250 ml water and left for 24 h. The sieves were removed from the bowls and the nematode suspensions poured into 500 ml nalgene bottles, adjusted to the fill level. After five hours, excess supernatant water was siphoned out with the aid of 3-cm inside diameter siphon tube inserted to the spout, until the siphon process breaks up automatically at a factory-fixed level just above the concentrated nematode suspension. Five 1 ml aliquots of the suspensions were observed under the stereomicroscope, and nematode populations counted. Final nematode population was determined by the addition of larval and egg populations for each treatment. Final and initial nematode populations were used to determine the reproduction factor of the nematode, using the formula, $R = P_f \div P_i$, where R is reproduction factor, P_i is initial population and P_f is final population.

At harvest, the remainning three replicates that constitute the second batch of the experiment were uprooted, following which number the of galls per plant were counted, and the seeds were obtained and weighed. All the data were collated and subjected to analysis of variance, using the general linear mode (GLM) procedure of the Statistical Analysis System version 8.2. The means were separated by least significant difference ($P \leq 0.05$).

RESULTS

Table 1 compares the host status of *S. indicum*, using Sasser *et al.* (1984) standard method of screening for crop resistance to root knot nematode infection with Afolami's (2000) intergrated method. There was similarity in host designations under both methods when considering E8 and NICRIBEN-01M, which were consistently tolerant and resistant irrespective of inoculum level. However, this is not true for Pbtil (No. 1) which was considered susceptible, using the known standard sytem of rating for resistance since this was shown to be tolerant to the nematode under the new system of rating that integrated grain yield assessment into the age long standard system. It is apparent that Pbtil (No. 1) was significantly ($P = 0.05$) damaged by the nematode root galls (index > 2) caused by *M. incognita* in addition to enhancing a 125–128% increase in population of the nematode. Hence, it is classified as susceptible using the Sasser *et al.* (1984) method. The universal prediction that a susceptible crop would have significantly lower yield when grown in soil with threshold population levels of the nematode pest was not sustained by this *S. indicum* breeding line. The risk of discarding this breeding line was averted by careful application of Afolami's (2000) modification of the age-long traditional scheme.

Table 1. A comparison of resistance designation of three *Sesamum indicum* varieties using the traditional galled index rating scheme of Sasser *et al.* (1984) and Afolami's integrated scheme (2000).

Genotype	Inoculum level	Resistance based on gall index +reproduction factor			Resistance based on gall index +yield % loss	
		Gall index (GI)	Reproduction Factor (R)	Resistance Designation	%Yield loss (YL)	Resistance Designation*
E8	5000	1	1.44	Tolerant	37.14 ns	Tolerant
	10000	2	1.63	Tolerant	54.97 ns	Tolerant
PbTil (No.1)	5000	3	1.28	Susceptible	4.12 ns	Tolerant
	10000	4	1.25	Susceptible	8.22 ns	Tolerant
NICRIBEN-01M	5000	1	0.26	Resistant	1.20 ns	Resistant
	10000	1	0.24	Resistant	2.40 ns	Resistant

*Resistance: $R \leq 1$, $GI \leq 2$, no significant yield loss; Tolerance: $R > 1$, $GI \leq 2$, no significan yield lost; Hypersusceptible: $R \leq 1$, $GI > 2$, significan yield loss; Susceptible: $R > 1$, $GI > 2$, significant yield loss.

Table 2 shows root knot disease assessment of *S. indicum* at mid-season and at harvest as well as grain yield production of Mi-infected plants. There was root knot nematode disease establishment in roots of all the *S. indicum* lines tested at both inoculum levels and on the two sampling dates when compared with the uninfested control. However, the disease was significantly higher at mid-season in Pbtil (No. 1), a susceptible genotype, when compared with root knot disease symptom at harvest. Although, the number of galls observed on the resistant and tolerant genotypes were higher at mid-season than at harvest, the plants either retarded the reproduction of the nematode or withstood the effect of the infection on grain production.

Table 2. A comparison of *Meloidogyne incognita*-induced root galls on three *Sesamum indicum* genotypes at midsaeson and harvest

Genotype	Inoculum level	Number of galls at midseason (Gall index)	Number of galls at harvest	Mean	±SD	Grain yield (g) per plant	One Hundred Grains weight (g) per plant
E8	0	0.00	0.00	0.00	0.00	1.71	0.32
	5000	2.00	0.33	117	1.18	1.06	0.31
	10000	5.33	2.33	3.83	2.12	0.77	0.22
Pbtil (No.1)	0	0.00	0.00	0.00	0.00	0.73	0.28
	5000	8.67	2.67	5.67	4.24	0.69	0.23
	10000	13.67	4.00	8.84	6.84	0.67	0.25
NICRIBEN-01M	0	0.00	0.00	0.00	0.00	1.67	0.29
	5000	1.33	0.67	1.00	0.47	1.65	0.25
	10000	1.67	0.33	1.00	0.95	1.63	0.27
Mean		1.33	0.89			1.18	0.27
±SD		1.33	0.87			0.45	0.03

DISCUSSION

Plant parasitic nematode control and management have remained a crucial issue throughout the world. Several well known methods and strategies have been adopted in developed and developing agriculture. Adesiyan *et al.* (1990) emphasised that the success and adoption of such methods depend largely on the level of expertise and socio-economic situation of the farmers. Management of nematodes 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.

Various remarkable attempts have been made by several scientists in the development and screening of nematode-resistant crop plants (Adesiyan *et al.* 1990). Methodologies for screening and communicating resistance in crops to *Meloidogyne* spp. have witnessed tremendous improvement over the past 48 years (Oostenbrink 1966; Cook 1974; Taylor and Sasser 1978; Canto-Saenz 1983; Sasser *et al.* 1984). Afolami (2000) reviewed Sasser *et al.* 's (1984) harmonisation using four soybean varieties as test crops and suggested a modification that would include yield loss indices in the well known standard gall and nematode reproduction indices, after comparing the efficacy of the old standard system with this new integrated method of Afolami (2000).

The ambiquity observed in Sasser *et al.*'s (1984) rating scheme was confirmed by Afolami (2001) when twenty rice varieties were screened for resistance to *M. incognita*. This provided further evidence that yield as the ultimate interest of the farmers, should be incorporated into the rating sheme in order to make such evaluation and selection of resistant cultivars more meaningful. In this way, poor yield performance of crop previously designated as resistant to *M. incognita* (Sasser *et al.* 1984) could be avoided. Furthermore, Afolami and Orisajo's (2003) screening work on rice, and Odeyemi's (2004) on cowpea both indicated the superiority of going beyong root galling as a measure of plant damage since gall index can only be a preliminary indicator of host reaction to the nematode. Consequently, Atungwu *et al.*'s (2003) screening of 14 accessions of *S. indicum* only offered prelimary information on the susceptibility or otherwise of the crop to *M. incognita*. The present study which adopted the recommendations of Afolami (2001) in evaluating the pathogenicity of *M. incognita* on three selected *S. indicum* lines lay further credence to the superiority of the new method over Sasser *et al.*'s (1984) method. The short-comings of the old system developed as an improvement over the various system used prior to 1984 has been comprehensively discussed by Afolami (2000, 2001).

Of the three *S. indicum* selected based on Atungwu *et al.* (2003, 2005) preliminary field and screen house studies that utilised Sasser *et al.* (1984) method which designated NICRIBEN-01M (syn: 530-6-1), E8 and Pbtil (No. 1) as resistant, tolerant and susceptible, respectively, two, including NICRIBEN-01M and E8 were consistent with earlier results in that the infected plants suffered no significant yield loss when compared with uninfected plants. NICRIBEN-01M did not only inhibit *M. incognita* reproduction by 74–76%, it also inhibited the nematode from causing significant damage to the roots of the plants, thus ensuring yields comparable with the uninfected control. Subsequently, it is designated as a truly resistant *S. indicum* variety. E8 is considered tolerant to *M. incognita* in this study, thus confirming the earlier works of Atungwu *et al.* (2003, 2005) as it allowed excellent (144–163%) reproduction of the nematodes in its roots without suffering significant reduction in grain yield. However, Atungwu *et al.*'s (2003, 2005) previous work and the present rating of Pbtil (No. 1) using Sasser *et al.*'s (1984) scheme as susceptible was nullified by Afolami's (2000) improved rating scheme.

Our current results as well as the previous works of Afolami (2000, 2001), Afolami and Orisajo (2003) and Odeyemi (2004) based on the new method tend to indicate that a resistant breeding line has triple advantage of (a) superior yield performance, (b) significant reduction of nematode population in the soil and (c) insignificant damage by the nematode. It was also observed that the root knot disease did not significantly affect the growth and grain yield. This study confirms Afolami's (2000) integrated method, thus upholding the efficacy of the method for studying, comparing and communicating resistance in arable crops to root knot nematodes.

It was apparent from this study that root knot disease decreased from mid-season to harvest season in all the *S. indicum* genotypes evaluated regardless of whether it was resistant, tolerant, or susceptible. The disease symptoms were significantly less evident in the 5000 inoculum treatment than the 10000 treatment across the varieties studied, in conformity with the linear correlation expected between nematode pests and the disease they cause.

This study has confirmed the resistant status of *S. indicum* variety, NICRIBEN-01M which when used in rotation with susceptible crops is capable of managing *M. incognita* infestation. Rotation strategies are usually based on resistance, susceptibility, or tolerance of crops to the predominant species of plant parasitic nematodes in a specific area. To be effective, the resistant crop component, as preceeding crop must necessarily prevent damage to the following crop by suppressing populations of the nematode pests with consequent improvement in the yield of the crop in order to justify its economy as a crop protection tactic. Based on Atungwu *et al.*'s (2003, 2005) and the current results, *M. incognita*-resistant *S. indicum* variety, NICRIBEN-01M is advocated as economic components of principal crops in rotation systems for sustainable management of the nematodes, especially for endemic areas in sub-Saharan Africa. Egunjobi (1992) opined that crop rotation is the oldest and effective nematode management option. Mechanism of resistance to *M. incognita* is being investigated to elucidate the inter-relationship between the nematode and *S. indicum*.

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POLISH SUMMARY

PATOGENICZNOŚĆ *MYLOINDOGYNE INCOGNITA* W STOSUNKU DO *SESAMUM INDICUM* I EFEKTYWNOŚĆ SCHEMATU OPARTEGO NA WYDAJNOŚCI W OCENIE ODPORNOŚCI

W porze deszczowej 2004 i 2005 r. przeprowadzono dwa doświadczenia pod osłonami mające na celu zbadanie reakcji trzech wybranych odmian *Sesamum indicum* na trzy gęstości populacji guzaka korzeniowego, *Meloidogyne incognita*. Rozsady *S. indicum* hodowano w doniczkach w układzie całkowicie zrandomizowanym i inokulowano 0, 5000 oraz 10000 jaj *M. incognita*, w sześciu powtórzeniach. Postęp choroby oceniano w połowie sezonu hodowlanego i podczas zbiorów. W celu oceny i opisu odporności na *Meloidogyne* ssp. zaadaptowano nową metodę, która dzieli procedurę klasyfikacji na dwie fazy w tym samym doświadczeniu. W pierwszej fazie reakcję badano tradycyjną, standardową metodą wykorzystującą tylko indeksy wyrośli i reprodukcji nicieni, natomiast w drugiej wzięto także pod uwagę wpływ choroby na produkcję ziarna w uprawie. Według obydwu podejść, tradycyjnego i ulepszonego, żywicieli E8 i NI-CRIBEN-01M (syn: 530-1-6) zgodnie zaklasyfikowano jako odpowiednio tolerancyjnego i odpornego. Jednakże, linia hodowlana *S. indicum* Pbtil (No. 1), uważana za podatną według starego systemu okazała się tolerancyjna według systemu zintegrowanego i ulepszonego. Liczba wyrośli na korzeniach powodowanych przez nicienie zmniejsza się począwszy od połowy sezonu hodowlanego do zbiorów. Użycie plonu jako dodatkowego parametru do oceny odporności roślin na guzaki korzeniowe uzupełnia obraz wzajemnego oddziaływania *Sesamum–Meloidogyne* z czego wynika przejrzystszy system określania reakcji rośliny żywicielskiej.