EFFECT OF ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) ON THE NODULATION OF SOME VARIETIES OF COWPEA (*VIGNA UNGUICULATA* L. WALP)

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Screenhouse and field trials were conducted at the University of Ilorin, Nigeria in 2013 and 2014 to investigate the effect of root knot nematode (*Meloidogyne incognita*) on the nodulation of five varieties of cowpea (IT89^{KD}-288, IT82^{D}-994, IT93^{K}-452-1, T89^{K}-391 and TT97 568-18). Half of the experimental plants were inoculated with *Meloidogyne incognita* eggs (n = 1500) while the remaining half served as uninoculated control plants. Data on growth and yield of cowpea, root gall, nodulation, and nematodes population densities in nodules were collected. Other parameters assessed included colour of dissected infected and uninfected nodules, texture of the nodules and varietal performance of the cowpeas. The results of both screenhouse and field trials were consistent in yield and visual observations of texture and colour. The yield of nematode-free varieties was significantly higher than that of *M. incognita*-infected ones. Infected nodules were dark brown in colour while the healthy ones were pink. For the field trials, nodulation counts in nematode inoculated plants were inversely proportional to the number of galls in their uninoculated counterparts in some varieties. Of all varieties, IT89^{KD}-288 was most resistant to *M. incognita.* Due to high cost involved in the management of nematodes, especially with synthetic chemicals, variety IT89^{KD}-288 is recommended for use in nematode endemic areas in Nigeria. Further study needs to be done on the histopathology of the infected and uninfected roots and nodules of these cowpea varieties.

inoculation, sodium hypochlorite, nitrogen-fixation, leghaemoglobin, root galls, screenhouse, field trial

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INTRODUCTION

Globally, cowpea (*Vigna uguiculanta* (L.) Walp) is a very valuable leguminous crop due to its nutritive value. It is a good source of protein and serves as a nutritional supplement to cereals in developing countries where cereal is a staple food. It is an important fodder which is also used for erosion control. In addition to being well adapted to the drier regions of the world, it can produce yield where other crops fail (Hall et al., 2002). Like all other legumes, cowpea has nodules on the roots. There occur *Rhizobium* spp. in the nodules, which are nitrogen-fixing bacteria that aid legumes to perform well in nitrogen deficient soil thereby enabling fertility restoration and hence could be used in amelioration of food security problems in areas prone to drought. However, cowpea is very susceptible to root knot nematode and suffers massive infections which have caused serious economic constraints in the production of the crop (Yakub, Izuogu, 2013). Studies have shown that plant parasitic nematodes alone or their interaction with some micro-organisms inhibit nodulation and nitrogen fixation in certain legumes (Siddiqu, Hussain, 1991, 1992). The importance of nodules in nitrogen fixation cannot be overemphasized and hence factors that mitigate their formation and innate potentials in leguminous plants must be checked. While de-emphasizing on the use of hazardous synthetic chemicals and owing to the tussle between human and pathogenic micro-organisms, man had continuously engaged himself in the development of resistant varieties to these organisms. Many cowpea varieties have been developed which are resistant to many diseases such as fungal, bacterial, viral and nematode but there is only a dearth of information on the effect of nematode on the nodulation of different cowpea varieties. This needs to be understood.

The present study was undertaken to evaluate the effect of *Meloidogyne incognita* on the nodulation of different cowpea varieties in relation to growth and yield of each variety.

MATERIAL AND METHODS

This experiment was carried out at the Crop Protection Screenhouse of the Faculty of Agriculture, University of Ilorin in September–November 2013 for screenhouse, and in August–October 2014 for field trials. The field is approximately 307 m a.s.l. and is located at the Southern Guinea Savannah Ecological Zone of Nigeria. The annual rainfall is 1250–1500 mm and the mean temperature range is 26–35°C. The soil is a well-drained sandy-loam. Five varieties of cowpea ($IT89^{KD}$-288, $IT81^{D}$-994, $IT93^{K}$-452-1, $T89^{K}$-391 and TT97-568-18) were purchased as a single batch from IITA (Ibadan, Nigeria).

Soil sterilization/field preparation

For pot trials, moist top soil was collected from the field and heated in a drum for 24 h to become sterilized and microorganisms-free. It was thereafter allowed to cool for another 48 h before distributing into 10-litre perforated buckets (Gowen et al., 2005). For the field, the soil was ploughed, harrowed and ridged for the experiment.

Experimental layout

The experimental design in screenhouse was 5 × 2 factorial fitted into a complete randomized design with each having 8 replicates, the field was 5 × 2 split-plot replicated four times. Three seeds of cowpea from each variety were planted per pot in September 2013 after which watering was done on a daily basis. Thinning of seedlings to one vigorous plant per pot was done at 7 days after emergence. The field measuring 35 × 20 m was divided into two plots (inoculated and uninoculated control) of 15 × 20 m with a 5 m × 20 m alley in-between them. Each plot was further sub-divided into four blocks with each block containing five sub-plots. While the blocks served as replicates, the sub-plots were the five varieties that either received *M. incognita* eggs (inoculated) or were not inoculated (control).

Extraction of root-knot nematode eggs

Heavily galled roots were uprooted from an established culture of *Celosia argentea* infected with *Meloidogyne incognita.* The extraction was done using the method described by Hussey, Barker (1973).

Procedure

The galled roots were gently washed under running tap and chopped into smaller pieces of about 2 cm; the chopped roots were poured into a 1-litre container. 5% sodium hypochlorite (NaOCl) was diluted in water at the ratio of 10 : 100 and added to cover the roots. It was then vigorously shaken for 4 min to digest the gelatinous egg matrix. Thereafter it was poured through a 200 mesh sieve nested over a 45 µm sieve and 25 µm sieve below to collect the freed eggs. The eggs on the 25 µm mesh sieve were gently washed with a cold stream of gentle tap water for 15 min to rinse off residual NaOCl. The eggs were then collected into a beaker as a suspension. The suspension was further diluted with water such that 1 ml contained 50 eggs.

Inoculation

For the inoculated plants, 50% in each experimental condition received the inoculum; 30 ml of the extracted nematode eggs suspension were inoculated per plant translating to approximately 1500 eggs per plant. The remaining 50% left uninoculated with *M. incognita* eggs either in the screenhouse or field served as control plots.

Data collection

Data on growth parameters such as plant height, number of leaves from the fourth week after planting were collected bi-weekly, while yield and yield related parameters, gall rating and nodulation count were recorded at harvest (12 weeks after planting). Visual assessments of nodules for sign of decay or morphological changes in colouration were done. Nodules were also cut open to assess the colour of infected and uninfected plants. Nematode population from soil was estimated using the modified Baermann's technique described by Whitehead, Hemming (1965). Infected nodules, which were decayed, were soaked in water and teased using a needle to release the nematodes, if present. The suspension was left for 6 h to allow the nematodes migrate out and settle. Thereafter, water was gently decanted and the suspension viewed under the compound microscope for identification.

Root gall rating

The root gall index was assessed on the scale of 0–10 as described by Bridge, Page (1980):

Table 1. Effect of root-knot nematode (*Meloidogyne incognita*) on the mean height of five cowpea varieties in pot and field trials

Varieties	Treatment	Pot				Field			
		weeks after planting							
		2	4	6	8	2	4	6	8
IT89^{KD}-288	inoculated	20.41^{ab}	26.50^{ab}	36.70^{a}	44.90^{ab}	22.30^{a}	30.80^{a}	42.60^{a}	51.20^{a}
	not inoculated	22.60^{a}	28.20^{a}	32.40^{b}	44.90^{ab}	17.30^{ab}	27.40^{abc}	37.80^{b}	53.40^{a}
IT81^{D}-994	inoculated	18.30^{ab}	24.40^{bc}	27.50^{e}	27.10^{d}	20.90^{ab}	26.30^{bcd}	29.00^{d}	29.70^{c}
	not inoculated	17.60^{ab}	23.20^{cde}	27.20^{e}	29.30^{d}	17.80^{ab}	27.10^{bcd}	29.30^{d}	31.00^{c}
IT93-451-1	inoculated	22.90^{a}	27.80^{ab}	31.80^{c}	36.60^{c}	22.60^{a}	29.00^{ab}	36.20^{bc}	37.60^{b}
	not inoculated	18.20^{ab}	23.80^{cd}	31.40^{bcd}	38.50^{bc}	17.10^{ab}	27.20^{bcd}	35.20^{bc}	41.60^{b}
IT89^{K}-391	inoculated	18.40^{ab}	22.90^{cde}	29.30^{bcde}	38.50^{bc}	17.40^{ab}	24.30^{cd}	35.50^{bc}	38.20^{b}
	not inoculated	16.70^{ab}	23.50^{cd}	31.50^{bcd}	41.40^{abc}	18.80^{b}	24.60^{cd}	37.90^{b}	42.70^{b}
IT97-568-18	inoculated	17.10^{ab}	20.70^{de}	28.60^{cde}	44.00^{ab}	16.80^{ab}	24.80^{cd}	33.30^{bcd}	50.10^{a}
	not inoculated	17.50^{ab}	19.80^{c}	28.00^{de}	47.30^{a}	16.00^{b}	23.60^{d}	32.40^{cd}	42.10^{b}
SE		1.02	1.15	1.15	2.05	0.01	1.12	1.54	1.99

SE = standard error of treatment means

Means in the same column followed by different letters are significantly different according to Duncan's multiple range test at $P < 0.05$

Table 2. Effect of root-knot nematode (*Meloidogyne incognita*) on the mean number of leaves of five cowpea varieties in pot and field trials

Varieties	Treatment	Pot				Field			
		weeks after planting							
		2	4	6	8	2	4	6	8
IT89^{KD}-288	inoculated	10.50^{abc}	18.90^{ab}	22.10^{bc}	23.90^{bc}	11.00^{abc}	13.70^{ab}	23.70^{abc}	25.40^{b}
	not inoculated	10.90^{abc}	20.30^{ab}	36.30^{a}	29.30^{ab}	8.60^{c}	13.40^{ab}	30.40^{a}	34.60^{a}
IT81^{D}-994	Inoculated	7.90^{abc}	17.80^{ab}	$17,60^{c}$	15.20^{d}	12.00^{a}	11.80^{b}	16.60^{c}	20.50^{c}
	not inoculated	11.00^{abc}	16.70^{b}	17.10^{c}	20.30^{cd}	9.30^{bc}	1.10^{ab}	17.00^{C}	22.70^{bc}
IT93-451-1	Inoculated	9.80^{abc}	18.00^{ab}	21.70^{bc}	24.40^{bc}	10.60^{abc}	12.00^{b}	20.90^{bc}	8.96^{cd}
	not inoculated	10.80^{abc}	17.90^{ab}	25.90^{bc}	19.90^{cd}	10.60^{abc}	12.90^{ab}	20.10^{bc}	23.65^{bc}
IT89^{K}-391	Inoculated	11.00^{abc}	20.90^{ab}	24.10^{bc}	24.00^{bc}	11.40^{ab}	14.40^{ab}	22.40^{abc}	28.20^{ab}
	not inoculated	11.40^{ab}	22.10^{a}	28.30^{ab}	36.40^{a}	11.80^{abc}	$15.70^{a`}$	26.70^{ab}	32.92^{a}
IT97-568-18	Inoculated	11.00^{abc}	20.90^{ab}	21.40^{bc}	26.70^{bc}	10.00^{abc}	15.30^{a}	23.30^{abc}	24.50^{bc}
	not inoculated	12.10^{a}	19.60^{ab}	30.40^{ab}	30.60^{ab}	11.00^{abc}	14.90^{ab}	24.90^{abc}	26.20^{b}
SE		0.72	1.49	3.08	2.40	0.71	0.98	2.52	2.95

SE = standard error of treatment means

Means in the same column followed by different letters are significantly different according to Duncan's multiple range test at $P < 0.05$

0 = no knots on roots; 1 = few small knots difficult to find; 2 = small knots only but clearly visible, main roots clean; 3 = some larger knots visible, but main roots clean; 4 = larger knots predominate but main roots clean; 5 = 50% of the roots knotted, knotting on parts of main root system; 6 = knotting on some of main roots; 7 = majority of main roots knotted; 8 = all main roots knotted, few clean roots visible; 9 = all roots severely knotted, plant usually dying; 10 = all roots severely knotted, no root.

Root nodule rating

The root nodule index was assessed on a scale of 0–5: 0 = no nodules, 1 = 1–10 nodules, 2 = 11–30 nodules, 3 = 31–50 nodules, 4 = 51–100 nodules, and 5 = above 100 nodules.

Analyses of data

At the end of the experimental trials, all numerical data collected were analyzed using the analysis of

Table 3. Effect of root-knot nematode (*Meloidogyne incognita*) on the mean number of pod, pod weight and seed weight of five cowpea varieties in pot and field trials

Varieties	Treatment	Pot			Field		
		pod number per plant	pod weight (g)	100-seed weight (g)	pod number per plant	pod weight (g)	100-seed weight (g)
IT89^{KD}-288	inoculated	11.00^{bc}	5.50^{ab}	21.50^{ab}	12.00^{a}	6.60^{abc}	22.40^{b}
	not inoculated	11.80^{b}	5.70^{a}	22.00^{a}	11.70^{a}	5.50^{bcd}	23.00^{ab}
IT81^{D}-994	inoculated	3.60^{c}	4.40^{c}	18.80^{d}	6.60^{d}	4.60^{d}	17.40^{a}
	not inoculated	9.80^{c}	5.10^{b}	20.50^{c}	7.10^{cd}	5.60^{bcd}	20.40^{c}
IT93-451-1	inoculated	11.00^{bc}	4.90^{bc}	18.50^{d}	9.40^{abc}	5.00^{cd}	18.80^{a}
	not inoculated	20.80^{a}	6.40^{a}	21.10^{b}	9.00^{bc}	6.90^{ab}	19.50^{cd}
IT89^{K}-391	inoculated	7.80^{a}	5.40^{ab}	21.30^{b}	10.10^{ab}	6.00^{abcd}	21.00^{bc}
	not inoculated	12.20^{b}	5.60^{a}	21.90^{ab}	9.70^{ab}	7.30^{ab}	24.20^{a}
IT97-568-18	inoculated	9.40^{c}	4.70^{bc}	20.30^{c}	9.70^{ab}	5.40^{bcd}	20.30^{c}
	not inoculated	12.40^{b}	5.40^{ab}	20.90^{bc}	11.00^{ab}	6.00^{abcd}	21.40^{bc}
SE		0.49	0.05	0.14	0.806	0.55	0.24

SE = standard error of treatment means

Means in the same column followed by different letters are significantly different according to Duncan's multiple range test at $P < 0.05$

Table 4. Effect of root-knot nematode (*Meloidogyne incognita*) on galls and nodules of five cowpea varieties in pot and field trials

Varieties	Treatment	Pot		Field	
		gall rating	nodule rating	gall rating	nodule rating
IT89^{KD}-288	inoculated	1.00^{b}	2.00^{b}	1.00^{b}	2.33^{c}
	not inoculated	0.00^{a}	3.70^{a}	0.20^{a}	4.25^{a}
IT81^{D}-994	inoculated	3.00^{c}	1.00^{d}	3.00^{c}	2.00^{c}
	not inoculated	0.00^{a}	2.00^{b}	1.00^{b}	3.63^{ab}
IT93-451-1	inoculated	4.00^{c}	1.70^{c}	4.50^{b}	1.72^{c}
	not inoculated	0.00^{a}	1.30^{cd}	0.80^{ab}	3.43^{b}
IT89^{K}-391	inoculated	6.00^{c}	1.00^{d}	5.00^{b}	2.00^{c}
	not inoculated	0.00^{a}	2.30^{b}	1.00^{d}	3.40^{b}
IT97-568-18	inoculated	2.00^{c}	1.30^{cd}	3.00^{c}	1.81^{c}
	not inoculated	0.00^{a}	2.30^{b}	0.30^{a}	3.64^{ab}
SE		0.18	0.01	0.14	0.21

SE = standard error of treatment means

Means in the same column followed by different letters are significantly different according to Duncan's multiple range test at $P < 0.05$

variance and the means were separated using the New Duncan's Multiple Range Test at $P = 0.05$.

RESULTS

Growth parameters such as plant height and number of leaves measured were not consistent between the nematode inoculated varieties and their control counterparts (Tables 1, 2). The yield of *Meloidogyne* uninoculated plants were generally higher than that of the inoculated ones (Table 3). The number of root galls were inversely proportional to the root nodules in most of the varieties (Table 4). Higher nodulation counts were observed in nematode uninoculated plants. Nodules of the uninoculated plants when cut open were strong and pinkish in colour, especially those from screenhouse, while the infected and decaying nodules from nematode inoculated plants were slightly soft, dark brown to black in colour. Root-knot nematodes were present in all the nematode inoculated plants and in three of the uninoculated ones from the field under the microscope including many dead ones from the decayed nodules. Variety IT89^{KD}-288 was moderately resistant to the effects of *M. incognita*.

The number of galls on the root of inoculated cowpea varieties varied while the control had no galls in all the pot trials and the field trial. All the other control plots in the field recorded very slight galling. Variety IT89^{KD}-288 also recorded a significantly higher number of nodules than the uninoculated ones.

DISCUSSION

Although a limited number of varieties were used for the study, results revealed that nodulation in nematode inoculated plants was inversely proportional to that in their uninoculated counterparts in some and not in all varieties. The implication is that varietal differences play an important role in nodulation with respect to their qualitative responses to nematode infection. Balasubramanium (1971) working with soybeans observed that when the nematode population is high, they interfere directly with the establishment of *Rhizobium japanicum* bacterium due to lowered production of root hairs in *Meloidogyne* spp. infected plants. A reduced number of nodules observed in the nematode inoculated plants may be due to the reduction of the cowpea root system as a result of root-knot nematode infection. The presence of *M. incognita* in the root nodules demonstrates varied levels of susceptibility of these cowpea varieties to root-knot nematode which were able to infect and possibly reproduce in them. It could also be deduced from the result that the development and reproduction of *M. incognita* in the root disrupted the physiological processes that depressed nitrogen-fixation by *Rhizobium* in the nodular tissues. The presence of black nodules which were decayed might indicate the absence of leghaemoglobin which is active in fixing nitrogen for the plants. Moran (1997) reported that legumes no longer fixing nitrogen turn green as a result of an inefficient *Rhizobium* strain or poor nutrition. The blackness of the nodules in the present study could be a result of dead cells and tissues and root galls. Though root nodule bacteria fix atmospheric nitrogen and have been reported to produce toxic metabolites inhibitory to many plant pathogens (Haque, Gaffar, 1993), the result of this study does not totally agree with the above report probably because of the initial high nematode inoculum used especially in the screenhouse trials. They possibly overwhelmed the inhibitory activities of the toxic metabolites released by *Rhizobium*. The initial inoculum density (1500 eggs of *M. incogita*) on hatching into second stage juveniles (J2) disrupted the physiological processes of the plant causing poor nodulation which resulted in suppressed activities of *Rhizobium* in the nodular tissue, thus reducing nitrogen fixation. The result agrees with earlier findings of Ali et al. (1981) who reported that rhizobia strains in leguminous hosts may lose their ability of nitrogen fixation especially when the hosts are infected by certain pathogens. The nematode was also able to suppress the activities of rhizobia especially in pot trials (sterilized soil) because of the absence of some other beneficial micro-organisms which in adequate population and in combination with *Rhizobium* promote growth of plants and reduce severity of disease. It has been reported that a single agent is not active in all soil nutrients or against all pathogens that attack the host plant (Akhtar, Siddiqu, 2008), but mixtures of biocontrol agents with different plant colonization patterns may be useful for the biocontrol of different plant pathogens via different mechanisms of different suppression. It is also possible that the diverse naturally existing micro-organisms in the field had synergized with the nodular *Rhizobium* and released some secondary metabolites which are antibiotic in nature as well as confer a level of resistance to the plants against the infection caused by *M. incognita*. The qualitative potentials of these varieties of cowpeas used played out in their response to yield and nematode infection. While variety IT89^{KD}-288 gave a better yield, it was closely followed by varieties IT93-45-1 and IT97-568-18. Variety IT93-451-1 is particularly noteworthy because it gave a relatively high yield despite its susceptibility to *M. incognita* as evidenced from the root galls and general reduction in growth. It could be proclaimed tolerant.

CONCLUSION

The cowpea varieties investigated differed in their susceptibility to *M. incognita* infection and nodule formation. Variety IT89^{KD}-288 exhibited a superior quality in terms of resistance to nematode and higher nodulation production. The following were the varieties IT93-45-1 and IT93-568-18 which were tolerant to *M. incognita* and gave a good yield. We therefore recommend varieties IT89-288, IT93-451-1 and IT97-568-18 for planting in *Meloidogyne* infested fields to ensure higher nodulation and tolerance to nematode infection. Their higher yield justifies our recommendation. Further studies should be intensified in identifying varieties of cowpea that are capable of forming high nodules and yielding well in root-knot nematode-infested field. This promises to be a very good management method that will blend well with cultural control.

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