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Novel Organic Fertilizers For Management of Root-Knot Disease of Soybean [*Glycine max* (L.) Merrill]

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Abstract
Organic farming is currently receiving great impetus in Africa due to its potentials to improve nutrition, income and livelihoods of resource-poor smallholder farmers. Sustainable management of root-knot nematodes in organically grown soybean remain a major challenge in Africa. Greenhouse studies were conducted to evaluate the efficacy of three novel organic fertilizers (sunshine, neem, and IAR & T fertilsers) as organic nematicides for control of Meloidogyne incognita (Mi) in a susceptible soybean variety. The organic fertilizers were applied two weeks before planting. All three organic fertilizers were significant (P < 0.05) in reducing (96 – 99 %) Mi nematode populations in soil leading to 86 – 100 % improvement in soybean grain yield. There were high and positive relationships between plant heights stem girth and yield components. Root gall development on test crop was negatively correlated with plant growth (r = - 0.47 to – 0.48) and yield (r = - 0.57 to – 0.59).

Keywords: *Correlation, Neem, organic farming, root-knot nematodes, soil amendments*

Introduction

Organic agriculture is relative recent in sub-Saharan Africa, but awareness of and the demand for organic produce has increased tremendously in the last two decades due to its potential to improve nutrition, income and livelihoods of smallholder farmers (Atungwu *et al*., 2009a). The guaranteed premium prices of organic food and sustainability of organic agroecosystem have continued to stimulate the interest of more people on the continent to grow and eat organic food.

However, pests and diseases including plant-parasitic nematodes pose great crop production constraints during the early years of conversion to and/or establishment of organic farms. Atungwu *et al*. (2009b) noted that Nigeria is the leading soybean producer in Africa. However, organic soybean production is seriously threatened by pests because organic standards do not permit the use of synthetic chemicals for pest control. The southern root-knot nematodes, *M. incognita* are well documented as a major limiting factor in soybean production in Nigeria (Adesiyan *et al*., 1990; Ihekwuemere, *et al*., 1996; Atungwu and Kehinde, 2008). Grain yield losses of up to 87% have been reported in nematode susceptible soybean grown in Nigeriaõs arable soils (Afolami and Atungwu, 2001). Organic soybean production excludes the use of synthetic nematicides which are often dangerous to both targeted and non-targeted and often beneficial organisms in the soil.

Therefore, to profitably grow soybean organically there is a need for development of acceptable, affordable and sustainable means of controlling the southern root-knot nematodes, *M. incognita* currently ravaging agricultural soils in Nigeria. Advocacy for organic farming in the country has led to the development of commercially available organic-based fertilizers by Government agencies and private organisations. These products which are often 350 - 500 % lower in prices compared to inorganic fertilizers are currently undergoing nation-wide trials for crop yield performance. Evaluating them for grain yield production alone may not sufficiently document the potential role of these novel organic inputs in curbing the incursion of Mi in organic soybean fields. . Although, Egunjobi (1992) recommended farmyard manure as effective nematicides for farmers in Nigeria, un-cured manure is not acceptable in organic farming, hence, the need to compost or process into organic fertilizers. Therefore, efforts of fertilizer companies to provide readily available and acceptable organic fertilizers warranted this study.

Up to 93% inhibition of nematode reproduction has been reported resulting in 10 times higher soybean grain yield in soil amended with organic materials (Atungwu and Lawal, 2008; Atungwu *et al*., 2009). Atungwu and Lawal (2008) tested an organic based fertilizer developed by the Institute for Agricultural Research and Training (IAR & T) at Ibadan in Nigeria and achieved considerable reduction in Mi nematodes in soybean. Atungwu *et al*. (2009) also noted that neem product applied singly or in combination with the IAR & T organic feriliser was equally efficient in inhibiting nematode reproduction in Mi-susceptible soybean. Atungwu and Kehinde (2008) earlier concluded that the combination was comparable with a synthetic nematicide, Furadan 3G in reducing Mi nematode population in TGx 1019-2EN soybean. In fact, according to these authors, organic fertilizer boosted soybean grain yield of the soybean over and above Furadan-treated plants. These levels of efficacy of organic materials against nematodes have compelled nematologists to evaluate gthe various organic fertilizers in the country for relative effectiveness. The objective of this study was to determine the efficacy of three novel organic based fertilizer formulations for control of *Meloidogyne incognita* for soybean production in Nigeria.

Materials and Methods

A screenhouse experiment was conducted in 2008, and repeated in 2009 at the University of Agriculture, Abeokuta, Nigeria. Three novel organic fertilizers were used namely, Sunshine organic fertilizer, Neem fertilizer, and IAR & T fertilizer. They were applied in soil in quantities of 40 g each per 4 kg of sandy loam topsoil which had been heat-sterilised at $65^{o}C$ for 1.5 hr. Overall; 4 kg of soil were used per 6-litre pot. The soil was homogeneously mixed with 1 % w/w of each of the organic fertilizer 14 days prior to planting. Pots that received no organic fertilizer served as control. The bottoms of the pots were perforated to allow drainage of excess water during daily watering.

Physiochemical properties of the soils were determined (Table 1) using routine methods such as flame photometer and Kjeldahl analytical procedure. Soybean variety, TGx1019-2EN was the study crop and seeds were sourced from the International Institute for Tropical Agriculture (IITA) at Ibadan in Nigeria. Proximate compositions of the organic fertilizers are presented in Table 2.

Treatments include:

a) Control: TGx 1019-2EN inoculated with *Meloidogyne incognita* + no fertilizer ;
b) TGx 1019-2EN inoculated with *Meloidogyne incognita* + sunshine fertilizer ;
c) TGx 1019-2EN inoculated with *Meloidogyne incognita* + IAR& T fertilizer; and
d) TGx 1019-2EN inoculated with *Meloidogyne incognita* + neem fertilizer .

Treatments were laid out in completely randomised design with six replications. The seeded pots were watered daily. Each pot was made to support one soybean plant

Inoculation of soybean seedlings

Pure culture of *M. incognita* previously maintained on susceptible *Celosia argentea* variety, TLV 8 obtained from the National Horticultural Research Institute at Ibadan in Nigeria, was used for inoculating soybean seedlings. Eggs were extracted from eight weeks old highly galled *C. argentea* roots, using 0.52% active ingredient (a.i.) sodium hypochlorite (NaOCl) method (Hussey and Barker, 1973). During extraction, washed galled roots of *C. argentea* were cut into 1ï 2 cm pieces and placed in a 200 ml of 0.52 % a.i NaOCl at the ratio of 1:3 in a 500 ml capacity conical flask. Then it was tightly corked and shaken manually but vigorously for three minutes to dissolve the gelatinous matrix enclosing the egg masses. The suspension containing the eggs was quickly poured over a 200-mesh sieve, sandwiched upon a 500-mesh sieve in which the eggs were caught. The 500- mesh sieve containing the eggs. was quickly placed under a gentle stream of cool tap water to rinse off residual of NaOCl. The rinsed *M. incognita* eggs were poured into a beaker after rinsing the 500-mesh sieve. Egg suspension was adjusted to 400 ml and calibrated by pipetting out 1 ml aliquot into a Doncaster (1962) ringed counting dish and the eggs were estimated under a stereomicroscope in the laboratory.

Each seedling was inoculated with 5,000 eggs of the nematode, one week after emergence. Inoculation was done by pouring suspension containing 5,000 eggs of *M. incognita* into a shallow circular trench around the base of the seedling (Iheukwumere *et al*., 1995). The trench was covered with small quantity of the topsoil after which it was wet lightly and daily afterwards.

Data collection and analysis

Data were obtained on plant height, stem girth and number of branches at 4 and 8 weeks after inoculation. Sixty days after inoculation, destructive plant sampling was done in 3 out of the 6 replicates per treatment to determine the number of galls and to estimate nematode reproduction ability. To achieve this, *M. incognita* eggs were extracted from soybean roots using Hussey and Barker (1973) sodium hypochlorite (1 %) method earlier described. Adult nematodes were extracted from the soil using Whitehead and Hemming (1965) method. The summation of the nematode eggs and adults produced the final nematode population, which was divided by the initial 5,000 egg population to estimate the reproduction potentials of the nematode.

Data collected were subjected to analysis of variance (ANOVA) using SAS (2000) software. Significantly different treatment means were separated using Fisherǿs Least Significant Difference ($LSD_{0.05}$).

Results and Discussion

Data for the effect of the study of organic fertilizers on key growth parameters of soybean grown in *M. incognita* infested soil are presented in Table 3. All the organic fertilizers had statistically significant ($P < 0.05$) positive influences on the growth and stem girths of soybean plants. Plant height ranged from 47.74 to 66.90 cm in fertilizer-treated plants at four weeks after inoculation (WAI), to 85.98 to 103.00 cm 8WAI in 2008. These growth responses were significantly ($P<0.05$) higher than their control counterparts. Similar responses were observed for stem girth in the fertilizer-treated plots (Table 3). Results obtained in 2008 were consistently parallel with those of 2009

The positive impacts of the fertilizers on growth of soybean plants, in spite of the initial aggressive Mi populations and the susceptibility status of TGx 1019-2EN soybean varieties was linked to the nematicidal effect of the fertilizers. In 2008, it was clearly shown that Mi population was effectively suppressed in the soybean roots and in the soil as a result of organic fertilizer application. Final population of Mi recovered from each plot varied significantly from fertilizer-treated plants to the control. Neem fertilizer gave the highest observed suppressive effect (99 %) on Mi population followed by IAR & T (98 %), and sunshine fertilizers (96 %) which were all similar but statistically ($p = 0.05$) different from the control treatment. Reduced Mi populations in the rhizosphere of the soybean plants significantly (p = 0.05) lowered the severity of the root-knot disease from 86 galls per plant in control treatment to 1 ï 5 galls per plant observed in roots of organic fertilizer-treated TGx 1019 soybean variety. This means that the fertilizers tested in this experiment suppressed root-knot disease by 94 ï 99 %. Neem gave the highest reduction in Mi nematode population and it subsequent damage on the soybean plants evaluated. The 2008 results were in concurrence with the 2010 observations.

The role played by the three organic fertilizers in suppressing southern root-knot nematode *M. incognita* population vis-à-vis reduction in plant damage by the invading nematodes could be directly linked to the marked increases in yield and yield components (Table 5) of the inoculated soybean plants grown in organic fertilizer-amended soil. Number of branches, pods and seeds per soybean plant varied significantly (p = 0.05) between amended soil and their control counterparts both in 2008 and 2010 experiments. There were 77 ï 86 %, 64 ï 71 %, 90 ï 106 % and 86 ï 100 % higher pods, seeds, pod weight and seed weight per plant respectively in soybeans plants that received organic fertilizer application compared to untreated plants observed in 2008. Similar results were documented when the experiment was repeated in 2010. These statistics follow the same pattern observed by previous findings (Atungwu and Lawal, 2008; Atungwu and Kehinde, 2008, Atungwu *et al*., 2009b) .

Correlation studies presented on Table 6 relates some soybean growth indices with root galling and Mi infection indices. Correlation coefficients (r) were generally negative but insignificant between root galls and plant height and stem girths irrespective of the year of the experiment was conducted. This implied that gall initiation was sufficiently inhibited to such an extent that less root-knot disease severity was observed which did not warrant stunted growth that is usually characteristic of TGx 1019-2EN being an Mi-susceptible soybean variety. Consequently, the relationship between root gall and seed production was positive and significant in 2008 (r = 0.59) and 2010 (r = 0.57)

Soil amendment with different types of organic fertilizers caused significant reduction in populations of root-knot nematodes and their damage thus resulting in enhanced tolerance to Mi and subsequent improved yields of the soybean. This finding is in agreement with several previous workersô reports (Kaplan and Noe, 1993; Sharma *et al*., 1997; Verma *et al*., 1997; DôAddabbo *et al*., 2000). Akhtar and Mahmood (1986) and Rodriguez-Kabana (1986) found that organic amendment with composted manure, oil cast of neem (*Azadirachta indica*), and castor (*Ricinus cummunis*) were potent in reducing populations of phytonematodes due to their low C:N ratio (6-10) and high ammonium nitrogen content. Our findings in this present work particularly in neem fertilizer-treated plots showed significant reduction of the in the number of *M. incognita*

(Mi) eggs/infective stage juveniles, qualitative root damage referred to as galls, soil nematode populations and the reproducibility of the invading nematodes in the roots of the Mi-susceptible soybean variety tested. This is probably due to the action of the active ingredient *Azadirachtin* as reported in neem products studied by Atungwu *et al*. (2009b). Similar impacts were observed in the two other organic fertilizers evaluated even though their efficacies on nematode survival and reproduction were numerically lower than in the neem fertilizer-treated plants, they generally exhibited significant control potentials on the population and reproduction ability of Mi in the present study. This substantiates Atungwu (2006) report that organic materials such as soil amendments have the potentials for suppressing plant-parasitic nematodes populations in the soil thus can improve growth and yield performance of Mi-susceptible host plants. It also bears some credence to Engunjobiǿs (1992) report that organic materials are effective nematicides for farmers in Nigeria.

Positive correlation between root gall and seed production observed in this study implied that organic fertilizer application conferred on TGx 1019 ï 2EN the status of Mi-tolerance as against its actual designation as susceptible variety (Afolami and Atungwu, 2001). It goes to show that resistance/tolerance may be reasonably boosted through application of organic nematicide. Enhancement of tolerance/resistance of crop varieties through organic soil amendment is being investigated.

Conclusion

It has been clearly shown that organic farming would play a significant role in the management of nematode pests of arable crops. Organic fertilizers are effective nematicides that are ecologically acceptable and farmer friendly low cost production inputs and should be encouraged in the emerging organic sector in Nigeria and other African nations. This has positive implications for sustainable development on the African continent.

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Table 1.Physiochemical properties of the of the soil used for the experiments

Parameters	**Proportionate value**	
	Experiment I	**Experiment II**
pH (H_2O)	5.8	5.6
% Organic Carbon	0.86	0.89
% Organic matter	1.59	1.60
Total N (%)	0.16	0.19
Available P (%)	7.2	8.1
Na Cmol/kg	0.66	0.63
Exchangeable bases (c mol kg^{-1})		
K	0.23	0.21
Mg	0.82	0.79
Ca	0.89	0.91
C.E.C	2.79	2.81
Physical properties (cmol kg^{-1})		
Silt (%)	13.00	12.92
Clay (%)	6.19	6.21
Sand (%)	89.01	89.07
Textural class	Sandy loam	Sandy loam

Table 2. Macro nutrient constituents of the three organic fertilizers used for the study

Organic Fertilizer	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Sunshine Fertilizer	3.50	1.00	1.20
IAR&T Fertilizer	1.28	0.96	0.36
Neem Fertilizer	7.00	7.00	6.00

Table 3. Effect of three novel organic fertilizers on growth parameters of nematode*Meloidogyne incognita*-infected TGx 1019-2EN soybean variety

Treatment	Plant Height (cm) 4WAI	Plant Height (cm) 8WAI	Stem girth (cm) 4WAI	Stem girth (cm) 8WAI
			Experiment I (2008)	
Control	30.42	62.80	0.48	0.58
Sunshine Fertilizer	66.90	103.00	0.53	0.81
IAR&T Fertilizer	65.03	100.67	0.53	0.73
Neem Feriliser	28.60	77.25	0.36	0.64
Treatment mean	47.74	85.98	0.48	0.69
LSD (0.05)	20.80	39.23	0.10	0.17
			Experiment II (2009)	
Control	34.13	52.31	0.45	0.60
Sunshine Fertilizer	68.17	112.21	0.53	0.90
IAR&T Fertilizer	66.51	112.57	0.51	0.74
Neem Feriliser	32.53	71.31	0.37	0.71
Treatment mean	50.34	87.10	0.47	0.74
LSD (0.05)	20.15	35.35	0.11	0.19

Table 4. Effect of three novel organic fertilizers on nematode *Meloidogyne incognita* populations in TGx 1019-2EN soybean variety

Treatment	No. of galls per plant	*Meloidogyne incognita* eggs/J2 populations recovered per plant	No. of *Meloidogyne incognita* adults recovered from the soil	Final population of *Meloidogyne incognita* per plant	Nematode reproduction Factor (Final population/5000
			Experiment I (2008)		
Control	85.53	6,781	471	7,252	1.45
Sunshine Fertilizer	4.65	79	46	125	0.03
IAR&T Fertilizer	5.41	81	35	116	0.02
Neem Feritiser	1.21	35	12	47	0.01
Treatment mean	24.2	1,744	141	1,885	0.38
LSD (0.05)	28.11	2,901.5	190.1	3,207	0.61
			Experiment II (2009)		
Control	78.21	5,509	511	6,020	1.20
Sunshine Fertilizer	5.34	73	35	108	0.02
IAR&T Fertilizer	5.93	91	41	132	0.03
Neem Feriliser	1.65	37	15	52	0.01
Treatment mean	22.78	1,427.5	150.5	1,578	0.32
LSD (0.05)	27.22	2,115.8	193.3	2,998	0.05

Table 5: Effect of three novel organic fertilizers on yield components of soybean variety inoculated with *Meloidogyne incognita*

Treatment	No. of branches per plant	No. of pods per plant	No. of seeds per plant	Pod weight (g/plant)	Seed weight (g/plant)
			Experiment I (2008)		
Control	3	22	42	8.18	5.11
Sunshine Fertilizer	3	41	69	16.82	10.06
IAR&T Fertilizer	3	39	71	15.74	10.20
Neem Feriliser	1	39	72	15.52	9.50
Treatment mean	2.50	35.25	63.50	14.07	8.72
LSD (0.05)	2	12	26	6.40	1.05
			Experiment II (2009)		
Control	2	21	42	7.14	6.81
Sunshine Fertilizer	3	44	87	14.45	10.12
IAR&T Fertilizer	3	41	85	14.53	10.17
Neem Feriliser	2	38	71	8.52	9.51
Treatment mean	2.50	36	71.25	11.16	9.15
LSD (0.05)	2	13	23	6.13	1.19

Table 6. Correlation among growth indices of *Meloidogyne incognita*-inoculated TGx 1019-2EN soybean variety

Parameter	Plant ht (cm)	Stem Girth (cm)	Gall No.	Eggs/J2	Soil Population	Final Population	RFactor	Pod No.	Seed No.	Pod wt	Seed wt
					Experiment I (2008)						
Plant ht (cm)	1.00										
Stem Girth (cm)	0.52	1.00									
Gall No.	-0.48	-0.36	1.00								
Eggs/J2	-0.56*	-0.22	0.73**	1.00							
Soil Population	-0.12	-0.02	0.59*	0.76**	1.00						
Final Population	-0.29	-0.06	0.68*	0.90**	0.97**	1.00					
RFactor	-0.30	-0.06	0.68*	0.90**	0.96**	0.99*	1.00				
Pod No.	0.40	0.24	0.39	0.58*	0.48	0.55	0.55	1.00			
Seed No.	0.49	0.28	-0.59*	0.73*	0.50	0.62*	0.62*	0.93**	1.00		
Pod wt	0.52	0.29	0.42	0.53	0.25	0.37	0.38	0.87**	0.91**	1.00	
Seed wt	0.50	0.28	0.35	0.41	0.11	0.22	0.23	0.79**	0.83**	0.98**	1.00
					Experiment II (2009)						
Plant ht (cm)	1.00										
Stem Girth (cm)	0.52	1.00									
Gall No.	-0.47	-0.42	1.00								
Eggs/J2	-0.54*	-0.21	0.71**	1.00							
Soil Population	-0.21	-0.12	0.57*	0.81**	1.00						
Final Population	-0.31	-0.04	0.59*	0.88**	0.86**	1.00					
RFactor	-0.29	-0.05	0.57*	0.95**	0.87**	0.91**	1.00				
Pod No.	0.39	0.26	0.36	0.66*	0.46	0.45	0.44	1.00			
Seed No.	0.45	0.29	-0.57*	0.69*	0.49	0.65*	0.65*	0.91**	1.00		
Pod wt	0.51	0.31	0.41	0.51	0.30	0.35	0.39	0.89**	0.89**	1.00	
Seed wt	0.49	0.24	0.39	0.37	0.18	0.29	0.15	0.81**	0.91**	0.93**	1.00

* Significant at 5 % level of probability ** Significant at 1 % level of probability