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Research Article
Management of Root-Knot Nematode, *Meloidogyne javanica* on African Yam Bean with Pig Excrements

[1]Christopher C. Onyeke, [1]Godswill C. Ajuziogu, [2]Simon C. Eze and [2]Kevin I. Ugwuoke

[1]Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria
[2]Department of Crop Science, University of Nigeria, Nsukka, Nigeria

Abstract
Screen house experiments were conducted to ascertain the effects of soil amendments with pig excrements/manure at different levels (0 (controls), 10, 20, 30, 40, 50 and 60 t ha^{-1}) on the management of *Meloidogyne javanica* at different inoculum densities (0 (controls), 2,000 and 4,000 eggs) on the African yam bean. Soil amendment with pig manure significantly ($p \leq 0.05$) improved on the vegetative growth of African yam bean plants infected with eggs of *M. javanica* when compared with infected plants but without amendment. The amendment also significantly ($p \leq 0.05$) suppressed the formation of root galls and egg masses and consequently recorded lower gall and egg mass indices as against the controls. The effect of soil amendments with pig manure on the management of *M. javanica* on test plants increased with increase in amendment levels. It was also observed that the infectivity of *M. javanica* increased at a higher inoculums density (4,000 eggs). Generally, it can be concluded that incorporation of pig excrement/manure in African yam bean fields infested with *M. javanica* would lead to improved growth of plants and also suppress nematode population.

Key words: Management, *Meloidogyne javanica*, African yam bean, pig excrements, manure

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Corresponding Author: Christopher C. Onyeke, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The African yam bean, *Sphenostylis stenocarpa* (Hochst Ex.A. Rich) Harms is a leguminous crop in the family Fabaceae cultivated in tropical Africa for its high nutritional values (Okigbo, 1973; Allen and Allen, 1981; GRIN., 2009). The seeds are rich in protein and amino acids and can compare with soybeans (Abbey and Berezi, 1988). The protein contents of the tubers are higher than those in potatoes and other tuber crops (Uguru and Madukaife, 2001). It is rich in minerals such as K, P, Mg, Ca, Fe and Zn (Edem *et al*.,1990). There is a high preference for the seeds in West Africa, while the East and Central Africans utilize the tubers (Potter, 1992; Nwokolo, 1996). A delicacy from the African yam bean is normally prepared and served during puberty rituals for young girls in Ghana (Klu *et al*., 2001), while in Ekiti State of Nigeria, a special African yam bean meal is also served during child-naming ceremonies (Potter, 1992). At Nsukka, Enugu State of Nigeria, the seeds could be eaten roasted or cooked with smoked cocoyam ('Echicha'), palm oil, oil bean, salt and pepper added to taste.

There is need for research into minor and lesser known legumes such as African yam bean because there is increasing demand on widely grown pulses for supplements in livestock feeds (Uguru, 1996) and are important as a cheap source of good quality protein (Nnanyelugo *et al*., 1985) in the growing food insecurity and starvation the world over.

Unfortunately, pests and diseases have on a very large scale contributed to poor crop yield and famine till date. Root-knot nematodes (*Meloidogyne* spp.) had been reported to contribute in no small measure to yield losses among legumes and other crops (Anwar *et al*., 2009; Ogaraku and Chhangani, 2010; Adegbite, 2011; Sumbul *et al*., 2015). Onyeke *et al*. (2013) reported up to 71.5% yield reduction in African yam bean as a result of *Meloidogyne incognita* infection.

Farmers over the years have relied heavily on chemical nematicides for the control of plant parasitic nematodes but their withdrawal especially the methyl bromide from markets because of environmental and health concerns necessitated the need for safer alternatives. The problem with chemical nematicides is that they are highly toxic to humans and animals, beneficial soil micro-organisms and the danger of contamination of ground water and even bioaccumulation by plants (Alam and Jairajpuri, 1990; Akhtar, 1991). The use of organic soil amendment such as animal manure for the management of plant parasitic nematodes in crop plants is worth while, as it does not only suppress nematode reproduction but also improves on the soil fertility and structure (Rodriguez-Kabana, 1986; Arden-Clarke and Hodges, 1988; Oka, 2010; Amulu and Adekunle, 2015). The present work was therefore conducted to ascertain the efficacy of pig excrement/manure in management of *Meloidogyne javanica* (MJ) on the African yam bean.

MATERIALS AND METHODS

Source of african yam bean seeds: Wholesome seeds of the African yam bean (TSS 11 and TSS 112) were sourced from the seed bank of the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria.

Source of pig droppings used for soil amendment: Pig droppings were collected from a piggery located at Odenigwe in the outskirts of the University of Nigeria, Nsukka. Droppings were completely dried under the sun and then ground into fine powder using Thomas Wiley Laboratory Mill Model 4, Arthur Thomas Company.

Maintenance and extraction of nematode eggs used for inoculation: The MJ population was maintained for up to 8 weeks on susceptible African yam bean accession (TSS 112) served as source of inoculum. Galled roots of the African yam bean were gently exhumed, washed in fresh tap water to remove soil particles and subsequently cut into bits of 1-2 cm. Nematode eggs were extracted from the galls by following the NaOCL-extraction method (Baker, 1985). Extracted eggs were washed into a graduated beaker and the volume made up to 1,000 mL with sterile distilled water. The egg suspension was calibrated such that 1 mL contained 2, 00 eggs; 20 mL of the egg suspension contained 4,000 eggs of MJ and 10 mL contained 2,000 eggs.

Screen house experiment: This experiment was carried out at the Department of Plant Science and Biotechnology Screen House, University of Nigeria, Nsukka (Latitude 06°86'39.6"N, longitude 007°41'20.4"E and altitude 433 m above sea level) from May-August, 2014. The experimental design was a 7×3 factorial in Completely Randomized Design (CRD) replicated ten times. The factors were 7 levels of pig manure rates: 0.0 (control), 10, 20, 30, 40, 50 and 60 t ha^{-1} and 3 nematode inoculum densities: 0 (control), 2000 and 4000 eggs per plant. Top garden soil collected from fallow land was sterilized in a large drum by heating to a temperature of 100°C for 3 h using fire wood and later air-dried for a period of 4 weeks before starting the study (Anderson and Ingram, 1989). The physical and chemical properties of the soil were determined according to standard methods (IITA., 1989).The textural class of the soil was sandy loam (48% coarse sand, 27% fine sand, 11% silt and 14% clay) pH (in H_20) 6.2, pH (in KCl) 5.6, OM 1.57%, BS 58.06%, N 0.098%, CEC 12.4 µ/100 g, Na 0.11µ/100 g, K 0.09 µ/100 g, Ca 4.8 µ/100 g, Mg 2.2 µ/100 g, exchangeable acidity 0.8 µ/100 g and P (in ppm) 13.06, aggregate stability 5.81%, bulk density 1.50 g cm^{-3}, total porosity 43.39% and water retention 33.56%. The soil was mixed thoroughly with powdered pig manure at different amendment levels (10, 20, 30, 40, 50 and 60 t ha^{-1}). The amended soils were poured into polyethylene planting bags of 26.5 cm diameter and 18 cm depth. Soils without amendments (0 t ha^{-1}) served as controls. The African yam bean (TSS 11) seeds were planted two per bag but later reduced to one before nematode inoculation. Two weeks after germination the polyethylene bags to receive 2,000 eggs were inoculated with 10 mL of the egg suspension while those to receive 4,000 eggs were inoculated with 20 mL of the egg suspension by pouring into a shallow trench created around the root tips of each of the test plants (Hussey and Boerma, 1981) and covered immediately with top soil (Goswami and Chenulu, 1974). Plants that were not inoculated with egg suspensions served as controls but received 20 mL of sterilized distilled water. The planting bags were arranged in a CRD with ten replications. Each bag was watered (200 mL) on a day interval basis. Six weeks after inoculation with nematode eggs, the following data were collected: Shoot fresh weight, shoot length, number of galls per root system, gall index per root system, number of egg mass per root system and egg mass index per root system. Root gall per egg mass index was determined using a rating scale, according to Taylor and Sasser (1978); 0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 = more than 100 galls egg^{-1} masses per root system. The number of egg mass was determined using Phloxine B (MP Biomedicals, LLC, USA) to stain the roots (0.15 g L^{-1}) for 15 min for easy counting (Daykin and Hussey, 1985).

Statistical analysis: The data recorded were analyzed statistically with Genstat for windows, version 3.2. Treatment means were separated using Least Significant Difference (LSD) at 5% level of probability. Regression analysis was performed to determine the relationship between number of galls and egg masses and their responses to increasing amendment levels using Microsoft excel chart wizard 2007.

RESULTS AND DISCUSSION

Results on the effect of soil amendment with pig manure on shoot fresh weight (g) and shoot length (mm) of SS inoculated with eggs of MJ are shown on Table 1 and 2. Results showed a significant ($p \leq 0.05$) increase in shoot fresh weight of SS as the tonnage of application increased. However, the least mean fresh shoot weight was recorded in inoculated and unamended controls. The highest mean fresh shoot weight was observed in the uninoculated but amended controls while the least mean fresh shoot weight was observed in plants amended but inoculated with 4,000 eggs. Also, significant ($p \leq 0.05$) differences were recorded between the amended controls and amended but inoculated plants and between the different inoculum densities.

Table 1: Effect of soil amendment with pig manure on shoot fresh weight (g) per plant of *S. stenocarpa* 6 weeks after inoculation with MJ eggs

	Pig manure rate (t ha^{-1})							
Inoculum density	0	10	20	30	40	50	60	Mean
0 eggs/plant (control)	8.52	13.20	14.22	16.00	20.84	22.64	23.80	17.03
2,000 eggs/plant	4.40	10.84	11.94	14.66	18.26	20.14	21.66	14.56
4,000 eggs/plant	2.44	6.12	9.94	11.96	12.78	14.22	15.94	10.49
Mean	5.12	10.05	12.03	14.21	17.29	19.00	20.47	

LSD (0.05) for comparing inoculum density (I) Means: 1.291, LSD (0.05) for comparing Pig Manure level (PM) Means: 1.973, LSD (0.05) for comparing IxPM (interaction) Means: 3.417

Table 2: Effect of soil amendment with pig manure on shoot length (mm) per plant of *S. stenocarpa* 6 weeks after inoculation with MJ eggs

	Pig manure rate (t ha^{-1})							
Inoculum density	0	10	20	30	40	50	60	Mean
0 eggs/plant (control)	162.60	178.00	186.00	225.00	235.60	255.00	263.00	215.02
2,000 eggs/plant	154.00	169.00	175.60	199.80	228.80	246.60	256.00	204.25
4,000 eggs/plant	125.60	163.40	170.60	191.20	205.80	225.60	233.40	187.94
Mean	147.40	170.10	177.40	205.30	223.40	242.40	250.80	

LSD (0.05) for comparing inoculum density (I) means = 16.28, LSD (0.05) for comparing Pig Manure (PM) level means = 24.87, LSD (0.05) for comparing IxPM (interaction) Means = 43.08

Table 3: Effect of soil amendment with pig manure on Gall Index (GI) and Egg Mass Index (EMI) per plant of *S. stenocarpa* 6 weeks after inoculation with MJ eggs

	Pig manure rate (t ha^{-1})							
Inoculum density	0	10	20	30	40	50	60	Mean
Gall index								
2,000 eggs/plant	4.00	3.00	3.00	3.00	1.60	1.40	1.20	2.45
4,000 eggs/plant	4.00	3.00	3.00	3.00	1.82	1.62	1.40	2.54
Mean	4.00	3.00	3.00	3.00	1.71	1.51	1.30	
Egg Mass Index								
2,000 eggs/plant	4.00	3.00	3.00	2.00	1.24	1.00	0.64	2.16
4,000 eggs/plant	4.00	3.00	3.00	2.64	1.84	1.60	0.84	2.43
Mean	4.00	3.00	3.00	2.32	1.54	1.30	0.74	

LSD (0.05) for comparing inoculum density (I) means (GI) = NS, LSD (0.05) for comparing Pig Manure level (PM) means (GI) = 0.3685, LSD (0.05) for comparing IxPM (interaction) means (GI) = 0.5212, LSD (0.05) for comparing inoculum density (I) means (EMI) = 0.2004, LSD (0.05) for comparing pig manure level (PM) means (EMI) = 0.3749, LSD (0.05) for comparing IxPM (interaction) means = 0.5302

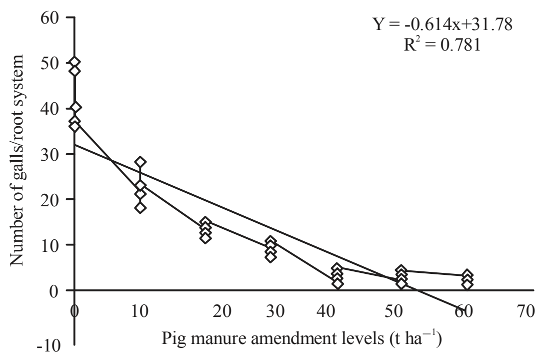

Fig. 1: Effect of increasing soil amendment with pig manure on number of galls per plant of *S. stenocarpa* 6 weeks after inoculation with MJ eggs

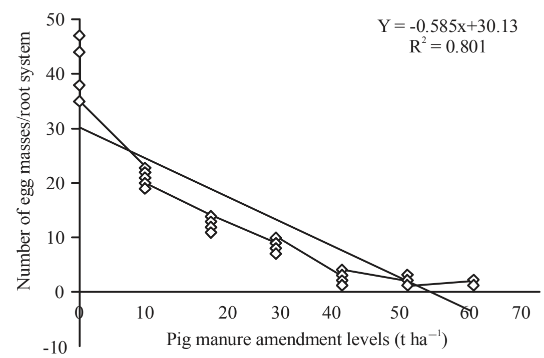

Fig. 2: Effect of increasing soil amendment with pig manure on number of egg masses per plant of *S. stenocarpa* 6 weeks after inoculation with MJ eggs

Results on the effect of soil amendment with pig manure on shoot length per plant of SS inoculated with eggs of MJ was generally similar to that of shoot fresh weight as shoot fresh length significantly ($p \leq 0.05$) increased as the rate of amendments increased. Also, the highest mean shoot length was observed in the uninoculated but amended controls, while, the least mean fresh shoot length was recorded in plants amended but inoculated with 4,000 eggs. This is an indication that the infectivity of MJ increased at a higher inoculum density (4,000 eggs). No phytotoxicity was observed for both growth parameters since plants recorded increase in growth even at the highest amendment level (60 t ha^{-1}).

These results are in agreement with an earlier reports of increased vegetative growth and yield of okra and tomato with the accompanying antinematode effect in a soil amended with animal manures (Nwanguma and Awoderu, 2002; Pakeerathan *et al*. 2009; Udo and Ugwuoke, 2010; Nwanguma *et al*., 2011). Babatola (1989) in a related experiment reported that increase in amendment level/rate of poultry manure translated to a better vegetative growth and yield of tomato crops infected with plant parasitic nematodes. The observed better vegetative growth of African yam bean plants amended with pig manure compared with the unamended plants could be ascribed to among other factors, the increase in nutrient supply from pig manure. Ultimately, organic amendments have been reported to contribute positively to the maintenance of soil structure, improvement of water retention capacity of the soil, enhanced crop rooting and availability of beneficial soil microflora and fauna which ultimately improves on nutrient availability in the soil (Arden-Clarke and Hodges, 1988). Similar report of increase in infectivity of MJ with increase in inoculum density has been recorded by Udo and Nwagwu (2007) and Ogaraku and Chhangani(2010) with tomato and cowpea, respectively.

Results on the effects of increasing soil amendment with pig manure on number of galls, number of egg masses and gall and egg mass indices are presented in Fig. 1 and 2 and Table 3. Results showed that organic amendment significantly ($p \leq 0.05$) reduced both the number of galls and egg masses per root system in test plants as against the unamended but inoculated plants. The number of galls and egg masses declined progressively as pig manure amendment levels inclined. The reduction in number of galls and egg masses per root system significantly ($p \leq 0.05$) and positively associated with increase in pig manure application levels ($R^2 = 0.781$ and 0.801, respectively). Results on the effect of soil amendment with pig manure on gall and egg mass indices per plant followed a similar trend with that observed in number of galls and egg masses. Abubakar *et al.* (2005) reported a significant ($p \leq 0.05$) drop in number of galls formed on tomato infected with MJ using a mixture of cow dung and urine. Also, Karmani *et al.* (2011) used a range of animal excreta as soil amendments and recorded significant ($p \leq 0.01$; 0.05) reduction in MJ infection in egg plants. Rizvi *et al.* (2015) observed significant ($p \leq 0.05$) drop in root gall formation, soil population of *M. incognita* and improved growth of lentil using composted cow dung alone and even better result when combined with *Rhizobium* sp. and other organic wastes(waste tea and egg shell). Also, Amulu and Adekunle (2015) reported that soil amendment with poultry manure or cow dung at the rate of 10 ha^{-1} could be effective in the management of root-knot nematode pest. Soil amendment with animal manure is a popular option in plant parasitic nematode management as highly nematicidal compounds are usually produced during decomposition process; also animal manure in the soil could affect the soil microfuana and flora in a way that enhances populations and activities of antagonistic micro-organisms to nematodes (Oka, 2010). Also, propagules of nematophagous fungi have also been reported to withstand the digestive enzyme activities of some ruminants such that amendment of soil with their excrements can readily be a source of antinematode fungal floral population (Wolstrup *et al.*,1996; Saumell *et al.*, 2000).

CONCLUSION

Conclusively, incorporation of pig excrement/manure in African yam bean fields infested with MJ would lead to improved growth of plants and also suppress nematode population.

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