Research Article

Management of Root-Knot Nematode (*Meloidogyne incognita*) in Okra (*Abelmoschus esculentus* L.) Moench) using *Trichoderma* Species and Poultry Manure

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Abstract | The screen house experiment was conducted to evaluate the efficacy of species of *Trichoderma* in combination with poultry manure in the control of *Meloidogyne incognita* infecting okra. *Trichoderma viride* at 2.65 x 10^7, 2.40 x 10^7, 1.85 x 10^7 spores/ml and T. *harzianum* at 2.40 x10^7, 1.40 x 10^7, 1.10 x 10^7 and 9.0 x 10^6 spores/ml were applied singly, in combination with poultry manure at 10, 15 and 20 t/ha. The obtained results showed highly susceptibility of okra to *M. incognita* with Gall index (G.I = 5.00) with no poultry manure amendment or *Trichoderma* infestation. Increase in poultry manure rates and spore density of *Trichoderma* applied singly were significantly (P< 0.05) reduced root galling, nematode density in soil per plant and nematode reproduction factor (R_f). The combination of different rates of poultry manure with either species of *Trichoderma* at various spore densities were significantly (P<0.05) inhibited root galling and nematode population more than single application. The combination of *T. viride* at 2.65 x 10^7 spores/ml or *T. harzianum* 2.40 x 10^7 spores/ml with 20 t/ha poultry manure produced okra with the least GI (2.00). The combination of *Trichoderma* species with poultry manure significantly (P<0.05) enhanced growth, dry matter accumulation and fresh pod yield.

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Introduction

Okra (*Abelmoschus esculentus* (L.) Moench) is a widely cultivated vegetable crop worldwide and very important in the diet of most Africans. It belongs to the family of Malvaceae (Omotoso, 2008). In Nigeria, it is widely grown, distributed and consumed in either fresh or in dried forms (Farinde, 2007). The West African region accounts for more than 75% of okra produced in Africa, but the average productivity in the region is very low (2.5t/ha) compared to East Africa (6.2t/ha) and North Africa (8.8t/ha) (FAOSTAT, 2006). Nigeria is the second largest producer of okra in the world after India and largest producer in Africa (5.8 million tons), followed by Cote d'Ivoire, Ghana and then others (FAOSTAT, 2011). Okra provides an important source of vitamins, calcium, potassium, carbohydrates and protein in large

quantities which are important in disease prevention (Adebayo and Oputa, 1996). The essential amino acids that okra contains are comparable to that of soybean making it a crucial part of human diet. The plant has immense medical and industrial values (Osawuru *et al*., 2011). Alcohol extract from the leaves can eliminate oxygen free radicals, improve renal function and reduced proteinuria (Siemonsma and Hamon, 2002). It has also been reported to prevent cancer and heart disease (Idu, 2009) as well as mucilage in midwifery (Obiere, 2002). Okra helps in lowering the blood sugar level by blocking the absorption of sugar in the intestinal tract.

The cultivation of okra is limited by biotic and abiotic stress factors. The poor yield of okra in Nigeria has been attributed in part to root knot disease caused by *Meloidogyne* species. The major limiting factor for its cultivation is the incidence of yellow vein mosaic virus which is transmitted by white fly. Fruits are mostly yellow, tough, small and fibrous once infected by this virus. Also, a number of insect pests like *Alphes gossypie, Thrips tabici* and *Helicoverpa armigera* are the deadliest major insect pests of okra (Osawuru *et al*., 2011). In order to improve the performance and yield of okra, there is need to find ways to combat these emerging pests through the use of different control measures such as biological, chemical and cultural methods. Although, chemical control of nematode pest remains the most effective control measure, there are some serious constraints. Chemical nematicides are very toxic to mammals and beneficial soil microfauna, pollute ground water and have residual effect on farm produce (Lohar, 2001). The use organic manure (poultry manure) and fungi (*Trichoderma* species) as a component of integrated nematode management is fast gaining wide acceptance (Faruk, 2019).

There are a number of approaches aimed at controlling root-knot nematodes through application of nematicides (Udo *et al*., 2014). Organic soil amendments from poultry droppings (Hussain *et al*., 2011), cultural management, physical and biological measures (Giannakou *et al*., 2019) have been advocated. Presently, researchers have diverted their attention to manage plant nematodes through the use of organic amendments and biocontrol agents such as fungi (Rao *et al*., 1997). On this note, the present study evaluated the effect of poultry manure and *Trichoderma* species on root-knot disease on okra.

Materials and Methods

Experimental site and source of material

This experiment was conducted in the screen house of the Department of Botany, University of Calabar. Okra seeds (*Clemson spineless*) VGTH-014K (Premier brand seeds) were obtained from National Institute for Horticultural Research (NIHORT) Ibadan, Oyo State. Poultry manure from Battery Cage System was obtained from Real Point Farm, Ikot Offiong Ambai, Akpabuyo Local Government Area, Cross River State.

Soil analysis for physicochemical properties and pre-nematode density

The composite soil sample (0-15cm) depth was subjected to routine soil analysis and also nematode population density was examined in the Soil Science Teaching and Research Laboratory, Department of Soil Science, University of Calabar.

Building up of nematode population/inoculum preparation

A pure stock culture of *M. incognita* was multiplied on susceptible Indian spinach (*Basella rubra*) plants in the screen house in a steam-sterilized sandy-loam soil. The nematode population was considerably built up at full maturity of the Indian spinach plants. The plants were slightly watered to soften the soil and thus allow for easy uprooting of the plants. The shoot system above the heavily galled root system was cut off and galled root properly washed with water to remove adhering soil particles. The gall root was then placed in moisten polythene bag and taken to the laboratory for inoculation preparation. The heavily galled roots of the Indian spinach plants were uprooted 8 weeks after planting and washed clean with tap water. The galled roots were cut into 1-2 cm segments for larvae extraction. The galled roots were then placed in a blender and distilled water added, blended at 5 seconds interval. The blended substrate was emptied into 1000 ml beaker and more water added and stirred. The suspension of 30 ml was poured into a nematode counting dish and the number of the larvae was counted with the use of a stereomicroscope. An average of such five counts gave approximately 5000 second stage juveniles per 30 ml of solution which was the inoculum density required.

Collection and culturing of Trichoderma species

The soil around the Piggery Farm of Faculty of Agriculture University of Calabar, Calabar and Zoo

Garden Mary Slessor Road, Calabar were collected using polyethylene bags, laboratory gloves, masking tape and trowel.

Sterilization of materials

The collected soil sample was taken to the laboratory in a sterilized polyethylene bags. The laboratory work bench was sterilized using sodium hypochlorite (bleach) solution and 99% of ethanol. The sterilization method used in this work was the wet steam method with an autoclave set at 160°C for 30 minutes.

Preparation of media for culturing of Trichoderma species

Potato Dextrose Agar (PDA) was used for culturing the fungus. The measured quantity of PDA (39 g) was poured into 1000 ml conical flask. 1000 ml of distilled water was added and stirred using magnetic stirrer until the solution gave a paste. Nonabsorbent cotton wool was covered with aluminum foil and used to cork the mouth of the conical flask which was rapped again with aluminum foil up to the neck of the flask. The medium was placed in an autoclave at 121 degrees Celsius for 15 minutes. The medium was allowed to cool before removing from the autoclave then 500 mg of Chloramphenicol was added to the solution and allowed to cool before pouring into labeled sterile Petri dishes.

Isolation of Trichoderma species

Soil samples collected were dropped in the plates containing PDA solution and labeled accordingly. The plates were incubated at room temperature of 27±1°C and daily observations were made for emergence of fungal colonies. Colonies formed were subculture to obtain pure cultures of the isolate.

Identification of the Trichoderma species

A drop of Lacto phenol in cotton blue was used to stain the slide. Sterilized inoculation needle was used to pick the spores of the fungi from culture plates and placed on the slide containing the lacto phenol in cotton blue then covered with cover slide for observation and identification under a light microscope (Olympus Optical Philippines) with magnification (x40). The morphological structures of the fungi were compared with those in the Atlas of Imperfect Fungi by Barnett and Hunter (1998) for identification.

Serial dilution of Trichoderma species

Sodium chloride (NaCl) of 0.85g was measured using Ohaus sensitive weighing balance and dissolved in 100 ml of distill water. The solution of (1ml) sodium chloride (NaCl) was diluted in (99ml) of distill water to give one percent (1%) sodium chloride (NaCl) solution in 250ml conical flask. This preparation was used as normal saline. A wire- loop (5 ml in diameter) of the *Trichoderma* species obtained from a 4-days old pure culture colony of fungi stock was diluted in one milliliter of one percent normal saline solution in 250 ml conical flask. Four test tubes in a test tube rack were labeled and arranged on the laboratory bench. The 9ml of normal saline was added to all test tubes. Automatic pipette was used to draw out one ml of the stock solution and added to the first test tube and mixed thoroughly using vortex mixer for one minute to have homogenous mixture which was the dilution concentration of 10^{-4} spores/ml of distilled water.

Another 1ml was transferred from the homogenous mixture of the first test tube into the second test tube and mix thoroughly to get dilution concentration of 10^{-6} spores/ml. This process was repeated for the remaining test tubes to get the dilution concentration of 10^{-8} spores/ml and 10^{-10} spores/ml respectively. One milliliter of the dilution solution of the last test tube was pipetted and discarded. This serial dilution process was done separately for the two species of *Trichoderma*.

Spore count of Trichoderma species

Spore count was done according to Pitt and Poole (1981) where a single chamber of hemacytometer (Neubabuer chamber) was used for the count. The spore concentration was determined as millions of spores per ml of sample and the number of spores generated was used for inoculation into bottles according to each treatment. The spores counted were as follows:

Trichoderma harzianum (T.h) 1×10^{-4} gave 2.40×10^{7} spores/ ml

Trichoderma harzianum (T.h) 1×10^{-6} gave 1.40×10^{7} spores/ml

Trichoderma harzianum (T.h) 1×10^{-8} gave 1.10×10^{7} spores/ml

Trichoderma harzianum (T.h) 1×10^{-10} gave 9.0×10^{6} spores/ ml

Trichoderma viride(T.v) 1×10^{-4} gave 2.65×10^{7} spores/ml

Trichoderma viride(T.v) 1×10^{-6} gave 2.40×10^{7} spores/ml

Trichoderma viride (T.v) 1×10^{-8} gave 1.85×10^{7} spores/ml

Trichoderma viride(T.v) 1×10^{-10} gave 1.05×10^{7} spores/ml

Preparation of carrier for Trichoderma species

Preparation of carrier for *Trichoderma* species was done according to Sivan *et al.* (1984). Five grams of millet grain was weighed using sensitive weighing balance. The millet was soaked in water for 24 hours to ferment before used. Normal sterilization of millet grains inside the bottles was done using autoclave at the range of 121°C for 15 minutes. The millet grains in the sterilized Bima bottles were allowed to cool before automatic pipette each indicating for *Trichoderma viride* and *Trichoderma harzianum* at different concentrations was used to draw out 1 ml of the spore count solution from the test tubes and inoculate into different designated bema bottle treatments containing sterilized substrates. Filter paper was used to cover the mouth of the bema bottles and the bored holes of the cover bottles were used to seal the mouth after inoculation. The substrates were taken immediately to screen house for inoculation into pots assigned treatments.

Poultry manure treatment, application and planting

Poultry manure was cured by using the in- house manure drying system on Slats method according to Elson and King (1975). The manure was kept for two weeks before applying on each pot receiving treatment. Ten tons per hectare (10t/ha), fifteen tons per hectare (15t/ha) and twenty tons per hectare (20t/ha) of poultry manure was used as the rate of application equivalent to 30, 50, and 60 grams per pot, respectively. Each pot was sown with three seeds of okra in 5kg of sterilized loamy sand and later thinned to one plant stand per pot at two weeks after planting (2WAP).

Nematode inoculation of potted okra plants

The pots were arranged on the screen house in a completely randomized design. Normal spacing of 30cm between rows and 30cm within rows was maintained. At 2WAP, the soil around the plant was excavated to the depth of 5cm and nematode suspension of 30ml (5,000 infective larvae) was inoculated into the soil. The soil around the roots was covered immediately.

Inoculation of potted okra plant using spores of Trichoderma species

The prepared millet grains carrier of 5 grams in Bima bottles having the inoculated Spores of *Trichoderma harzianum* and *Trichoderma viride* were taken to screenhouse for inoculation into pots. Holes were made in the soil some distance away from the okra plant. Inoculation of both species of *Trichoderma* was done at different spore concentrations in all the okra pots receiving treatments except the un-inoculated ones which served as control. The inoculated area was covered with soil immediately after inoculation.

Experimental layout, data collection and statistical analysis

The experiment was laid down in completely randomized design (CRD) having thirty-six treatments and three replications. Data were collected at two weeks interval starting from three weeks after planting (3WAP) on the following: Number of nematodes per plant, number of galls per root system, gall index, nematode reproduction factor, nematode density in 5kg of soil, plant height, number of leaves per plant, days to flowering, photosynthetically active radiation per plant, number of pods per plant, fresh pod weight per plant, mean weight of pod, fresh root weight per plant, dry leaf weight per plant and dry stem weight per plant. Data collected were subjected to analysis of variance (ANOVA) and significant means were separated using Duncan New Multiple Range Test (DNMRT) at 5% probability level.

Results and Discussion

Effects of poultry manure (PM) amendment and Trichoderma species inoculation on gall index (GI) of okra plants inoculated with Meloidogyne incognita

Table 1 shows okra plant (*Clemson spineless)* was highly susceptible to *Meloidogyne incognita* infection in pots without poultry manure as the gall index was rated 5.00. Poultry manure (PM) amendment generally reduced the susceptibility rating of okra plants from highly susceptible (G.I= 5.00) to susceptible (G.I= 4.00). Infestation of *Trichoderma viride* alone at the highest spore concentration of 2.65×10^7 spores/ ml (V1) reduced the susceptibility rating to moderately susceptible (G.I= 3.00) and this was the trend when both species of *Trichoderma* were combined with different rates of poultry manure. However, the combination of the highest spore load of either species of *Trichoderma* 2.40×10^7 spores/ml (H1+P3) and 2.65×10^7 spores/ml (V1+P3) with the highest rate of poultry manure 20 t/ha (P3) changed the susceptibility rating (G.I= 2.00).

Table 1 shows increase in poultry manure rate significantly ($P\leq0.05$) increased fresh root weight of okra plants. However, soil infested with 1.85×10^7 or

Table 1: *Effects of poultry manure and Trichoderma species on different parameters of okra plant inoculated with Meloidogyne incognita.*

Treatment rates/ concentration	Gall index	Plant height (cm)**	Number of leaves	Fresh root weight (g)/ plant	Fresh pod weight (g)	Dry leaf weight/ plant(g)	Dry stem weight/ plant (g)
P_0 (control)	5.00±0.00^{a}	7.67±0.333^{P}	3.67±0.333^{k}	11.03± 0.285^{tu}	15.20±1.752^{t}	1.843±0.0498^{t}	1.543±0.262^{t}
P_1 (10 t/ha)	4.00±0.00^{b}	9.33±0.333^{mno}	5.00±0.00^{ghi}	12.40±0.364^{s}	34.78±1.044^{p}	3.753±0.0657^{q}	4.567±0.294^{p}
P_2 (15 t/ha)	4.00±0.00^{b}	11.17±0.167^{jk}	5.67±0.333^{efg}	15.60±0.666^{pq}	39.87±1.616^{o}	4.107±0.0698^{op}	5.907±0.295^{o}
P_3 (20 t/ha)	4.00±0.00^{b}	12.17±0.167^{hi}	6.34±0.333^{cde}	18.28±0.513^{n}	48.23±1.737^{mn}	4.900±0.0964^{mn}	7.983±0.0376^{m}
H_1(2.40×10^7 spores/ml)	3.67±0.333^{c}	10.17±0.167^{l}	4.67±0.333^{hij}	14.73± 0.260^{q}	26.67±1.271^{q}	2.213±0.0441^{s}	2.503±0.227^{rs}
H_2(1.40×10^7 spores/ml)	4.00±0.00^{b}	10.00±0.00^{lm}	4.34±0.333^{ijk}	12.82±0.216^{rs}	19.20±1.770^{rst}	2.073±0.0410st	2.187±0.0876^{s}
H_3(1.10×10^7 spores/ml)	4.00±0.00^{b}	9.00±0.00^{no}	4.34±0.333^{ijk}	11.02±0.145^{tu}	19.99±0.948^{rs}	2.043±0.0406st	1.620±0.193^{t}
H_4 (9.0×10^6 spores/ml)	4.00±0.00^{b}	8.67±0.333^{o}	4.00±0.00^{jk}	10.24±0.312^{u}	18.41±0.768^{rst}	1.987±0.0176st	1.397±0.176^{t}
V_1 (2.65×10^7 spores/ml)	3.00±0.00^{d}	11.33±0.333^{jk}	5.34±0.333^{fgh}	15.92±0.174^{op}	29.29±0.616^{q}	2.727±0.0694^{r}	4.027±0.0561^{q}
V_2 (2.40×10^7 spores/ml)	4.00±0.00^{b}	9.67±0.333^{lmn}	4.34±0.333^{ijk}	13.62±0.266^{r}	22.14±1.199^{r}	2.347±0.0606^{s}	2.967±0.107^{r}
V_3 (1.85×10^7 spores/ml)	4.00±0.00^{b}	9.67±0.333^{lmn}	4.67±0.333^{hij}	11.82±0.232st	18.03±0.917^{rst}	2.233±0.0882^{s}	2.467±0.0726^{rs}
V_4 (1.05×10^7 spores/ml)	4.00±0.00^{b}	8.67±0.333^{o}	4.00±0.00^{jk}	10.88±0.0726^{tu}	17.47±0.924st	2.097±0.0606st	2.160±0.757^{s}
H_1+ P_1	3.00±0.00^{d}	12.17±0.167^{hi}	6.00±0.00^{def}	17.73±0.249^{n}	62.40±1.774^{k}	4.410±0.105^{o}	9.197±0.318^{l}
H_2+ P_1	3.00±0.00^{d}	11.17±0.167^{jk}	5.67±0.333^{efg}	16.63±0.293^{op}	55.40±1.019^{l}	4.093±0.0636^{op}	7.987±0.0689^{m}
H_3+ P_1	4.00±0.00^{b}	11.17±0.167^{jk}	5.34±0.333^{fgh}	16.73±0.217^{o}	48.31±1.287^{mn}	3.830±0.0666^{pq}	6.947±0.0902^{n}
H_4+ P_1	4.00±0.00^{b}	9.83±0.167^{lm}	5.34±0.333^{fgh}	15.69±0.260^{opq}	44.99±1.063^{n}	3.600±0.0577^{q}	6.313±0.104^{o}
V_1+ P_1	3.00±0.00^{d}	13.17±0.167^{fg}	6.67±0.333^{bcd}	21.89±0.310^{jk}	65.03±2.707^{k}	5.857±0.148^{k}	10.910±0.121^{j}
V_2+ P_1	3.00±0.00^{d}	12.17±0.167^{hi}	6.34±0.333^{cde}	20.72±0.319^{lm}	60.91±0.914^{k}	5.263±0.0684^{l}	9.933±0.161^{k}
V_3+ P_1	3.67±0.333^{c}	11.17±0.167^{jk}	6.00±0.00^{def}	19.80±0.156^{m}	51.45±1.077^{m}	4.733±0.0726^{n}	9.110±0.0985^{l}
V_4+ P_1	4.00±0.00^{b}	10.83±0.167^{k}	5.67±0.333^{efg}	25.69±0.180n	50.38±0.896^{m}	4.233±0.0882^{o}	8.173±0.0722^{m}
H_1+ P_2	3.00±0.00^{d}	13.83±0.167^{ef}	7.00±0.00^{bc}	25.69±0.641^{hi}	82.41±1.190^{efg}	6.600±0.176^{j}	13.273±0.241^{ef}
H_2+ P_2	3.00±0.00^{d}	12.50±0.289^{ghi}	6.34±0.333^{cde}	22.14± 0.491^{j}	74.27±1.730^{ij}	5.923±0.0788^{k}	12.007±0.0762^{h}
H_3+ P_2	3.00±0.00^{d}	11.83±0.167^{ij}	6.00±0.00^{def}	20.94±0.362^{kl}	71.24±0.927^{j}	5.400±0.153^{l}	11.033±0.142^{ij}
H_4+ P_2	3.00±0.00^{d}	11.17±0.167^{jk}	6.00±0.00^{def}	19.87±0.164^{m}	64.07±2.128^{k}	5.083±0.0726^{lm}	10.143±0.0536^{k}
V_1+ P_2	3.00±0.00^{d}	15.33±0.333^{c}	7.34±0.333^{b}	31.63±0.895^{e}	92.72±2.075^{b}	8.580±0.191^{ef}	15.047±0.111^{d}
V_2+ P_2	3.00±0.00^{d}	14.17±0.167^{de}	6.67±0.333^{bcd}	27.88±0.415^{g}	86.46±0.575^{de}	8.040±0.0569^{g}	13.570±0.229^{e}
V_3+ P_2	3.00±0.00^{d}	14.17±0.167^{de}	6.34±0.333^{cde}	26.22±0.383^{h}	79.13±1.006^{gh}	7.267±0.120^{hi}	12.663±0.0984^{g}
V_4+ P_2	3.00±0.00^{d}	12.83±0.167^{gh}	6.34±0.333^{cde}	25.03±0.120^{i}	71.05±0.875^{j}	6.967±0.0751^{i}	11.433±0.203^{i}
H_1+ P_3	2.00±0.00^{f}	16.17±0.167^{b}	7.34±0.333^{b}	32.10±0.276^{e}	92.13±1.460^{bc}	9.387±0.178^{c}	15.663±0.235^{c}
H_2+ P_3	2.33±0.333^{e}	15.17±0.167^{c}	6.67±0.333^{bcd}	30.07±0.105^{f}	85.04±0.729^{def}	8.777±0.0784^{de}	14.887±0.0928^{d}
H_3+ P_3	3.00±0.00^{d}	14.83±0.167^{cd}	6.34±0.333^{cde}	28.46±0.219^{g}	81.99±0.878^{fg}	8.023±0.0536^{g}	13.540±0.155^{e}
H_4+ P_3	3.00±0.00^{d}	14.33±0.333^{de}	6.00±0.00^{def}	27.55± 0.318^{g}	76.46±1.168^{hi}	7.433±0.117^{h}	12.823±0.184^{fg}
V_1+ P_3	2.00±0.00^{f}	17.50±0.289^{a}	8.34±0.333^{a}	39.50±0.454^{a}	106.43±2.470^{a}	10.787±0.368^{a}	17.590±0.135^{a}
V_2+ P_3	2.33±0.333^{e}	16.17±0.167^{b}	7.34±0.333^{b}	36.74±0.321^{b}	95.98±1.271^{b}	9.873±0.153^{b}	16.283±0.249^{b}
V_3+ P_3	3.00±0.00^{d}	14.33±0.167^{de}	6.67±0.333^{bcd}	35.34±0.285^{c}	88.33±1.448^{cd}	9.030±0.0361^{d}	14.857±0.0606^{d}
V_4+ P_3	3.00±0.00^{d}	13.67±0.333^{ef}	6.34±0.333^{cde}	33.90±0.103^{d}	80.41±0.739^{gh}	8.333±0.136^{fg}	13.153±0.264^{efg}

**Means within a column followed by the same letter of alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at 5% probability level. **Plant height; three weeks after planting (3WAP), P= poultry manure; V= Trichoderma viride; H = Trichoderma harzianum.*

1.05×10^7 spores/ml (V3 or V4) of *Trichoderma viride* and 1.10×10^7 or 9.0×10^6 Spores/ml (H3 or H4) of *Trichoderma harzianum* species produced okra plants that had similar fresh root weight with the control plants (Po). Increase in the spore load of both *Trichoderma* species led to a significant increase in fresh root weight. The combination of either species of *Trichoderma* at a given spore load with poultry manure at a specified rate produced okra plants with significantly higher fresh root weight than individual control agent at that given rate or spore load. The combination of all spore concentrations 2.65×10^7 to

1.05×10^7 spores/ml (V1+P3 to V4+P3) of *Trichoderma viride* with 20 t/ha poultry manure (P3) produced plants with significantly (P≤0.05) heavier fresh roots than the other treatments. However, (V1+P3) *Trichoderma viride* at 2.65×10^7 spores/ml in combination with 20 t/ha poultry manure (P3) produced okra plants with the heaviest fresh root weight.

Effects of poultry manure (PM) amendment and Trichoderma species inoculation on the plant height (cm) of okra inoculated with Meloidogyne incognita

Table 1 shows that at three weeks after planting (3WAP), poultry manure amendment and inoculation of either species of *Trichoderma* significantly (P≤0.05) enhanced the growth of okra plants compared with the control. Increase in the application rate of poultry manure led to a corresponding significant (P≤0.05) increase in height. In most cases, there was no significant (P>0.05) difference in height between plants inoculated with the two species of *Trichoderma* at a given spore load. However, there was a significant enhancement in okra plant height when poultry manure was combined with either species of *Trichoderma* compared with individual application of the control agents at given rate of poultry manure application or inoculum load of *Trichoderma species*. The results at 5 and 7 WAP followed the trend of 3WAP. However, at 5WAP, plants inoculated with 9.0×10^6 Spores/ml of *Trichoderma harzianum* (H4) were similar in height with the control plants (P0) and the same was true for those inoculated with 1.10×10^7 spores/ml (H3) and 9.0×10^6 Spores/ml *Trichoderma harzianum* (H4) and 1.05×10^7 Spores/ml of *Trichoderma viride* (V4). At all sampling periods, inoculation with 2.65×10^7 Spores/ml of *Trichoderma viride* (V1+P3) in combination with 20 t/ha (P3) of poultry manure amendment produced significantly (P≤0.05) the tallest plants.

Effects of poultry manure (PM) amendment and Trichoderma species inoculation on the number of leaves of okra inoculated with Meloidogyne incognita

Table 1 shows that at three weeks after planting (3WAP), application of poultry manure significantly (P≤0.05) enhanced leaf production in okra plants compared with the unamended control (P0). In contrast, infestations of soil with lower inoculum load of 1.85×10^7 to 1.05×10^7 Spores/ml (V3 to V4) of *Trichoderma viride* and of *Trichoderma harzianum* 1.10×10^7 to 9.0×10^6 spores/ml (H3 to H4) did not (P>0.05) enhance leaf production compared with the uninfected soil. The combination of either species of *Trichoderma* with successive increase in the rate of poultry manure amendment significantly increased the number of leaves produced when compared with individual application of each control agent. At 5 and 7WAP, higher rates of poultry manure (15t /ha and 20 t/ha) significantly enhanced leaf production compared with the control. Infestation of soil with either species of *Trichoderma* alone irrespective of the inoculum load produced plants with similar or less number of leaves relative to the control. However, the combination of the *Trichoderma* species with 15 t/ha (P2) or 20 t/ha (P3) of poultry manure led to a significant enhancement in leaf production compared with the control plants. Infestation of soil with *Trichoderma viride* at 2.65×10^7 spores/ml (VI+P3) in combination with 20 t/ha (P3) of poultry manure significantly produced plants with the highest number of leaves closely followed by *Trichoderma harzianum* at 2.40×10^7 Spores/ml (HI+P3) in combination with the highest rate of poultry manure.

The effects of poultry manure (PM) amendment and soil infestation with Trichoderma species on the leaf and stem dry weights of okra plants inoculated with Meloidogyne incognita

Table 1 shows that with the exception of lower inoculum load of both species of *Trichoderma,* all the treatments significantly (P≤0.05) enhanced leaf dry matter accumulation in okra plants relative to the control. Successive increase in the poultry manure rate significantly (P≤0.05) increased leaf dry weight. The combination of poultry manure with either species of *Trichoderma* consistently increased leaf dry weight of okra compared with individual application of the control agents at a given rate. However, *Trichoderma viride* at 2.65×10^7 Spores/ml (V1) in combination with 20 t/ha poultry manure (P3) produced plants with significantly the highest leaf dry matter. Results of the effects of treatments on dry stem weight followed the trend of leaf dry weight. However, *Trichoderma viride* infestation at all inoculum loads significantly (P≤0.05) enhanced stem dry matter accumulation compared with control. *Trichoderma viride* at 2.65×10^7 Spores/ml (V1+P3) in combination with 20 t/ha poultry manure significantly (P≤0.05) produced okra plants with the highest stem dry weight.

The effects of poultry manure (PM) amendment and Trichoderma species inoculation on fresh pod weight (g) of okra inoculated with Meloidogyne incognita

Table 1 shows increase in poultry manure rate

significantly (P≤0.05) increased okra fresh fruit yield compared with the control. Infestation of soil with highest spore load of either species of *Trichoderma* alone (H1 and H2) significantly (P≤0.05) enhanced fresh fruit yield of okra plants relative to the control, while lower spore load produced similar fresh fruit yield as the control. Combinations of poultry manure at various rates with the different spore load of either species of *Trichoderma* led to a significant (P≤0.05) fresh fruit yield increase compared with individual application at the specified rates of poultry manure or spore load of *Trichoderma* specie. However, the combination of the highest rate of poultry manure 20 t/ha (P3) with the highest spore load of *Trichoderma viride* (V1+P3) produced plants with the highest fresh fruit yield (P≤0.05). There were no significant (P>0.05) differences in fresh fruit yield among plants treated with *Trichoderma viride* at 2.65×10^7 spores (V1) in combination with 15 t/ha poultry manure (P2), *Trichoderma viride* 2.65×10^7spores/ml (V1) and *Trichoderma harzianum* at 2.40×10^7 spores/ml (H1) both in combination with 20 t/ha poultry manure (P3).

Effects of poultry manure (PM) amendment and Trichoderma species inoculation on number of galls per root system of okra plants inoculated with Meloidogyne incognita

Figure 1 shows that soil amendment with poultry manure significantly (P≤0.05) reduced root galling by the nematode species with the highest rate of poultry manure P3 (20t/ha) recording the least number of galls. Soil infestation with both species of *Trichoderma* with the least inoculum load 1.10×10^7 to 9.0×10^6 spores/ml (H3 to H4) and 1.85×10^7 to 1.05×10^7 spores/ml (V3 to V4) recorded okra stands with significantly (P≤0.05) fewer galls than poultry manure amendment with the highest rate P3(20 t/ha). The increase in inoculum load for both species of *Trichoderma* resulted in a significant (P≤0.05) increase in root galls comparatively. The highest spore concentration of *Trichoderma viride* (V1) had plants with significantly fewer numbers of galls than that of *Trichoderma harzianum* (H1). The combination of poultry manure with *Trichoderma* species always resulted in a significant (P≤0.05) decrease in the number of galls except in the case of 1.10×10^7 spores/ml with 10t/ha (H3+P1), 9.0×10^6 spores/ml with 10t/ha (H4+P1) and 1.05×10^7 spores/ml with 10t/ha (V4+P1). However, both *Trichoderma* species at their highest spore concentrations in combination with highest rate 20 t/ha of poultry manure (P3) produced okra plants with the least number of galls.

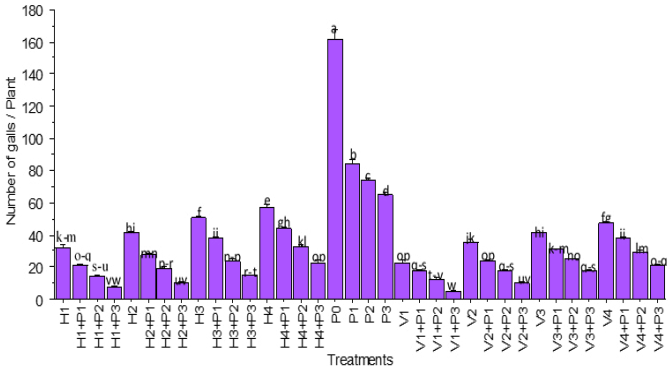

Figure 1: *Effect of treatments on number of galls/plant.*
Key: *Po = Poultry manure (Control), P1= Poultry manure 10 t/ha, P2 = Poultry manure 15 t/ha, P3= Poultry manure 20 t/ha, H1= Trichoderma harzianum 2.40×10^7 spores/ml, H2= Trichoderma harzianum 1.40×10^7 spores/ml, H3= Trichoderma harzianum 1.10×10^7 spores/ml, H4= Trichoderma harzianum 9.0×10^6 spores/ml, V1= Trichoderma viride 2.65×10^7 spores/ml, V2= Trichoderma viride 2.40×10^7 spores/ml, V3= Trichoderma viride 1.85×10^7spores/ml, V4= Trichoderma viride 1.05×10^7 spores/ml.*

Effects of poultry manure (PM) amendment and Trichoderma species inoculation on number of nematodes per plants inoculated with Meloidogyne incognita

Figure 2 shows that nematode density per okra plant was significantly (P≤0.05) reduced with increase in the poultry manure (PM) rates and spore concentration of both *Trichoderma* species compared with the control. However, at each spore load, *Trichoderma viride* 2.65×10^7 to 1.05×10^7 spores/ml (V1 to V4) gave a lower nematode density (P≤0.05) relative to *Trichoderma harzianum* 2.40×10^7 to 9.0×10^6 spores/ml (H1 to H4) infested plants. The combination of either species of *Trichoderma* with Poultry manure always produced plants with significantly (P≤0.05) lesser number of nematodes compared with individual application rates (control). However, plants infested with *Trichoderma viride* at spore load of 2.65×10^7 spores/ml (V1+P3) in combination with the highest poultry manure rate of 20 t/ha (P3) produced the least number of nematodes (P≤0.05) closely followed by the same species at 2.40×10^7 spores/ ml (V2+P3) at the same poultry manure rate 20 t/ha (P3) and then *Trichoderma harzianum* at 2.40×10^7 spores/ml (H1+P3) in combination with 20 t/ha (P3) poultry manure.

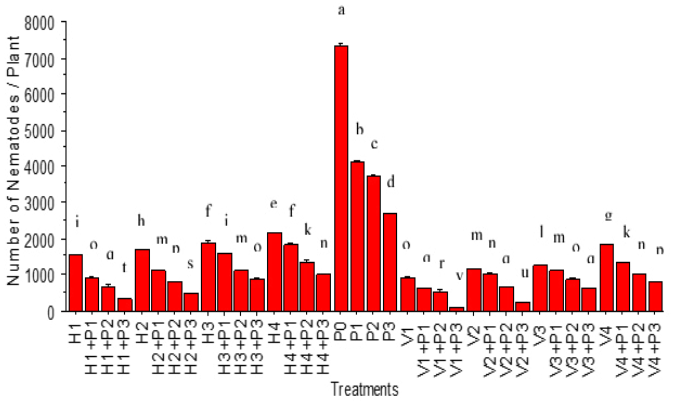

Figure 2: *Effect of treatments on number of nematodes/ Plant.*

Key: *Po= Poultry manure (Control), P1= Poultry manure 10 t/ha, P2= Poultry manure 15 t/ha, P3= Poultry manure 20 t/ha, H1= Trichoderma harzianum 2.40×10⁷ spores/ml, H2= Trichoderma harzianum 1.40×10⁷ spores/ml, H3= Trichoderma harzianum 1.10×10⁷ spores/ml, H4= Trichoderma harzianum 9.0×10⁶ spores/ml, V1= Trichoderma viride 2.65×10⁷ spores/ml, V2= Trichoderma viride 2.40×10⁷ spores/ml, V3= Trichoderma viride 1.85×10⁷ spores/ml, V4= Trichoderma viride 1.05×10⁷ spores/ml*

Effects of poultry manure (PM) amendment and Trichoderma species inoculation on nematode density in soil inoculated with Meloidogyne incognita

Figure 3 shows that the nematode population in the soil after harvest almost followed the trend of that in the plant. With the exception of 10 t/ha poultry manure rate, poultry manure amendment significantly (P≤0.05) reduced nematode number in the soil compared with the control experiment. Similarly, infestation of okra plants with both *Trichoderma* species alone significantly reduced soil nematode population. However, in both species of *Trichoderma*, increase in inoculum load significantly (P≤0.05) reduced nematode number in the soil. *Trichoderma viride* significantly reduced more number of nematodes in the soil than *Trichoderma harzianum* at each respective inoculum load. Combination of poultry manure with either species of *Trichoderma* resulted in a significant (P≤0.05) decrease in soil nematode population when compared with individual control agent at any given rate of application. However, the least number of nematodes in the soil was obtained when the soil was amended with 20 t/ha poultry manure and infested with 2.65×10⁷ spores/ml of *Trichoderma viride* (V1+P3), *Trichoderma harzianum* at 2.40×10⁷ spores/ml (H1+P3) combined with 20 t/ha poultry manure gave the same number of nematodes in the soil as *Trichoderma viride* at 2.40×10⁷ spores/ml combined with 20 t/ha poultry manure amendment.

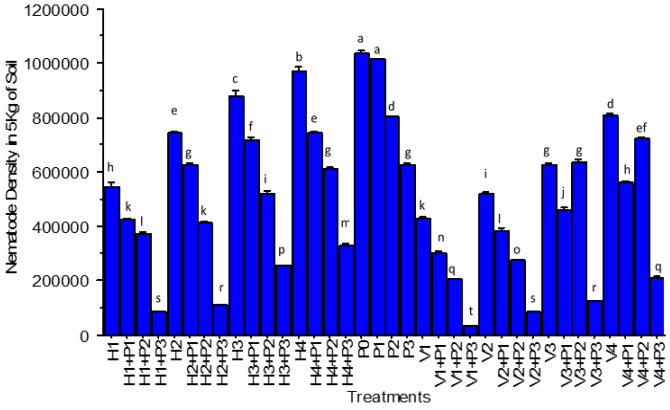

Figure 3: *Effects of treatments on nematode density in 5Kg of soil.*

Key: *Po = Poultry manure (Control), P1 = Poultry manure 10 t/ha, P2 = Poultry manure 15 t/ha, P3= Poultry manure 20 t/ha, H1 = Trichoderma harzianum 2.40×10⁷ spores/ml, H2= Trichoderma harzianum 1.40×10⁷ spores/ml, H3= Trichoderma harzianum 1.10×10⁷ spores/ml, H4= Trichoderma harzianum 9.0×10⁶ spores/ml, V1= Trichoderma viride 2.65×10⁷ spores/ml, V2= Trichoderma viride 2.40×10⁷ spores/ml, V3= Trichoderma viride 1.85×10⁷ spores/ml, V4= Trichoderma viride 1.05×10⁷ spores/ml.*

Effects of poultry manure (PM) amendment and Trichoderma species inoculation on nematode reproduction factor (R_f) inoculated with Meloidogyne incognita

Figure 4 shows the nematode reproduction factor (R_f) was significantly (P≤0.05) higher in the control experiment (no poultry manure amendment and no *Trichoderma* species inoculation), 10 t/ha poultry manure application and *Trichoderma harzianum* at 9.0×10⁶ spores/ml (H4) inoculation than other treatments. Higher rates of poultry manure amendment; 15 t/ha (P2) and 20 t/ha (P3) significantly (P≤0.05) reduced reproduction factor (R_f). Increase in the concentration of spores of both *Trichoderma* species significantly (P≤0.05) inhibited root-knot nematode reproduction in okra plants. However, at each respective spore load, *Trichoderma viride* significantly inhibited nematode reproduction more than *Trichoderma harzianum.* The combination of either species of *Trichoderma* with poultry manure at a given rate of application was in most cases more efficient (P≤0.05) in impeding nematode reproduction than individual application of the control agents. However, *Trichoderma viride* at 2.65×10⁷ to 1.85×10⁷ spores/ml (V1+P3 to V3+P3) in combination with 20

t/ha poultry manure and *Trichoderma harzianum* at 2.40×10^7 to 1.40×10^7 (H1+P3 to H3+P3) combined with 20 t/ha poultry manure had the least soil nematode density (P≤0.05).

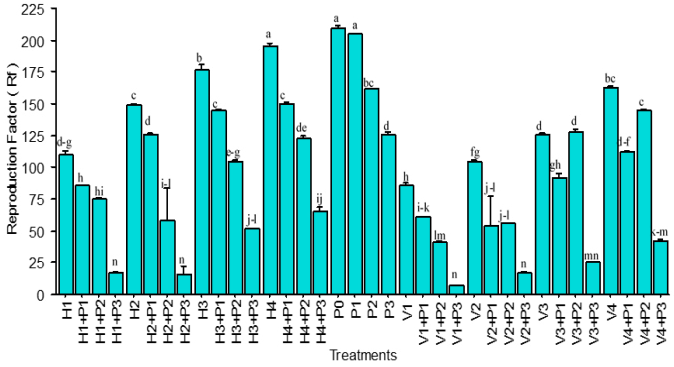

Figure 4: *Effect of treatments on nematode reproduction factor (R_f).*

Key: *Po = Poultry manure (Control), P1 = Poultry manure 10 t/ha, P2 = Poultry manure 15 t/ha, P3= Poultry manure 20 t/ha, H1= Trichoderma harzianum 2.40×10^7 spores/ml, H2= Trichoderma harzianum 1.40×10^7 spores/ml, H3= Trichoderma harzianum 1.10×10^7 spores/ml, H4= Trichoderma harzianum 9.0×10^6 spores/ml, V1= Trichoderma viride 2.65×10^7 spores/ml, V2= Trichoderma viride 2.40×10^7 spores/ml, V3= Trichoderma viride 1.85×10^7 spores/ml, V4= Trichoderma viride 1.05×10^7 spores/ml.*

The okra variety (*Clemson spineless*) used in this study was found to be highly susceptible to *Meloidogyne incognita* with gall index of 5.00 in soils without poultry manure and uninfested with either species of *Trichoderma*. This observation is in line with the report of Nwanguma (2002). Severe galling on the roots of okra plants by *Meloidogyne incognita* could be attributed to a conducive soil environment as nematodes activity is generally enhanced in sandy soils as reported by Agu (2008) and Olewe (2005). Increase in the rate of poultry manure led to a significant reduction in root galls, nematode density in both soil and okra plant and the reproduction factor (Rf) compared with soils without amendment. Generally, application of organic amendments either of plant or animal origin has been reported by many authors to suppress nematode population in the soil as well as their damage potentials (Nwanguma, 2002; Radwan *et al.*, 2007; Oka, 2010; Renco, 2013). Various mechanisms have been advanced for the possible effects of organic amendments on the tripartite interaction of nematode–plant soil system (Oka, 2010; Mcsorley, 2011; Renco, 2013). The release of preexisting nematicidal constituents, generation of nematicidal compounds such as organic acids, ammonia, nitrogenous compounds during decomposition, enhancement or stimulation of nematode antagonist, increase in plant resistance/ induced tolerance by microorganisms or natural compounds and changes in the physicochemical properties of the soil are some of the possible mechanisms listed by these authors. However, it could be very difficult to distinguish the most important mechanisms as they may operate simultaneously (Akhtar and Malik, 2000). It is possible that some of these mechanisms could have been involved in the suppression of *Meloidogyne incognita* population in this study. The poultry manure used in this work had a very narrow C: N of 8.95. Agu (2007) had observed that organic materials with low C: N ratios and high protein or amine type of nitrogen content were more effective in nematode suppression than those with high C: N ratios. In this study therefore, nematodes could have been killed through the release of nitrous acid which is reported to be more potent in acidic soils than ammonia as a product of decomposition (Oka, 2010). The highest nematode suppression was obtained with 20 t/ha poultry manure amendment which corroborates with the work of Nwanguma and Awoderu (2002) who reported high nematicidal activity of chicken litters against *Meloidogyne incognita* at 16 t/ha amendment rate comparable to a synthetic nematicide mocap.

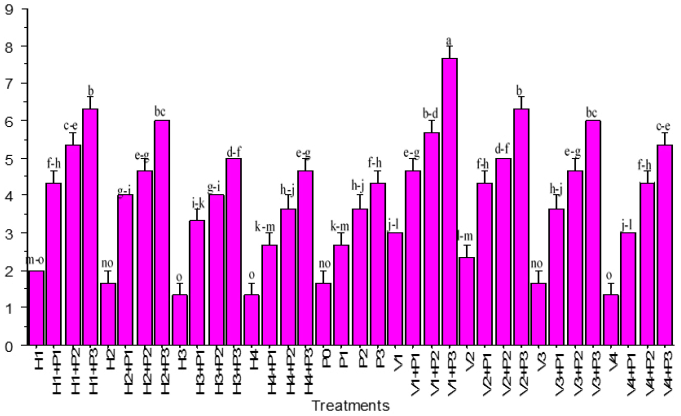

Figure 5: *Effect of treatments on number of Pods/Plant.*

Key: *Po = Poultry manure (Control), P1 = Poultry manure 10 t/ha, P2 = Poultry manure 15 t/ha, P3= Poultry manure 20 t/ha, H1= Trichoderma harzianum 2.40×10^7 spores/ml, H2= Trichoderma harzianum 1.40×10^7 spores/ml, H3= Trichoderma harzianum 1.10×10^7 spores/ml, H4= Trichoderma harzianum 9.0×10^6 spores/ml, V1= Trichoderma viride 2.65×10^7 spores/ml, V2= Trichoderma viride 2.40×10^7 spores/ml, V3= Trichoderma viride 1.85×10^7 spores/ml, V4= Trichoderma viride 1.05×10^7 spores/ml.*

Effects of poultry manure (PM) amendment and Trichoderma species inoculation on number of pods/ plant inoculated with Meloidogyne incognita.

Figure 5 shows that amendment of soil with poultry manure significantly (P≤0.05) enhanced fruit setting by okra when compared with the control experiment. With the exception of the highest spore load of *Trichoderma viride*, infestation of soil with either species of *Trichoderma* alone did not significantly (P>0.05) increase the number of pods produced by okra plants relative to the control. In most cases, the combined application of poultry manure and infestation with *Trichoderma* produced plants that significantly (P≤0.05) had more number of pods than individual application of the control agents. However, the highest spore load (2.65×10^7 Spores/ml) of *Trichoderma virde* in combination with the highest rate 20 t/ha of poultry manure (V1+P3) produced plants that significantly (P≤0.05) had the highest number of fruits followed by the highest spore load (2.40×10^7 spores/ml) of *Trichoderma harzianum* at the same poultry manure rate (H1+P3) as well as *Trichoderma viride* at 2.40×10^7 spores in combination with 20 t/ha poultry manure (V2+P3).

Infestation of soil with *Trichoderma harzianum* or *Trichoderma viride* alone significantly inhibited root galling, nematode density in both soil and okra with a decrease in nematode reproduction factor. The highest spore density was the most potent for both species of *Trichoderma*. These findings validate the work of previous researchers (Windham *et al*., 1989; Sharon *et al*., 2001; Sahebani and Hadavi, 2008). Similarly, Spiegel and Chet (1998) reported that the application different isolates of *Trichoderma harzianum* and *Trichoderma lignorum* caused significant reduction in *Meloidogyne javanica* population and root galling and enhanced the growth of infested plants in Israel.

Different mechanisms of biocontrol activity of *Trichoderma* species against many soil borne pathogens have been suggested which include; antibiosis, competition, mycoparasitism, enzymatic hydrolysis and induced systemic resistance (Sahebari and Hadavi, 2008). Penetration of eggs and colonization of *Meloidogyne javanica* juveniles have been illustrated *in vitro* by Sharon *et al*. (2001). Khan and Saxena (1997) illustrated the nematicidal/nematotoxic properties of the metabolites of *Trichoderma viride* used as root dip treatment on tomato against *Meloidogyne javanica* where nematode penetration and reproduction were adversely affected. Sharon *et al*. (2001) demonstrated the involvement of proteinase in the parasitic behavior of *Trichoderma* species on *Meloidogyne javanica* and they suggested that improvement in the proteolytic activity of the antagonist may be very vital for the biological control of plant parasitic nematodes. It is likely that one or more of the mechanisms mentioned above could have been deployed by the *Trichoderma* species used in this study to suppress the population of *Meloidogyne incognita* on okra plants with, *Trichoderma viride* being more efficacious in reducing root galling and nematode reproduction than *Trichoderma harzianum*. This may partially be attributed to its ability to sporulate faster as observed during culturing. It may suggests that *T. viride* is capable of exploiting the substrate used as carrier more than *T. harzianum* for its higher suppression activity on *Meloidogyne incognita* population. It could also be that its proteolytic activity is more than that of *T. harzianum* as suggested by Sharon *et al*. (2001).

The combination of either species of *Trichoderma* with poultry manure was more potent in reducing root galling and suppressing nematode population than individual application of the nematode control agents. This observation confirms the findings of earlier workers (Ehteshamul-Haque *et al*., 1996; Rao *et al*., 1998; El-Sherif and Ismail, 2009). Soil amendments with organic matter have been reported to simulate soil food webs, thus increasing the population of free-living nematodes, plant growth promoting rhizobacteria, fungi and nematode antagonist (Oka, 2010; Mcsorley, 2011). The role of the organic amendment could be complementary as it may help the antagonist like *Trichoderma* species to establish in the soil. The highest root gall reduction and suppression of *Meloidogyne incognita* population was obtained with the highest spore density of *Trichoderma viride* (2.65×10^7 spores/ml) and *Trichoderma harzianum* (2.40×10^7 Spores/ml) in combination with the highest rate of poultry manure (20 t/ha) indicating that the combination of various mechanisms of nematode control by the two agents may likely account for this observation. Amendment of soil with poultry manure and its combination with various spore densities of both species of *Trichoderma* significantly enhanced growth, dry matter accumulation, photosynthetic rate, earliness to anthesis and fruit yield of okra. These observations could be attributed to the reduction in root galling as against severe galling obtained in soils not amended with poultry manure or infested with

Trichoderma species. Root galling by *Meloidogyne incognita* is reported to impair water and nutrient uptake, translocation and photosynthesis (Williamson and Gleason, 2003). The poultry manure could have acted as a reservoir of plant nutrients slowly released to okra plants during its growth phase and fruiting stage, as the soil used for the trial was deficient in major plant nutrients. Udo and Ugwuoke (2010) reported increased growth and yield of turmeric (*Curcuma longa* L.) infected with *Meloidogyne incognita* by poultry manure amendment at the rate of 20 t/ha. They cautioned against higher rate greater than 20 t/ha to be phytotoxic. In the present study, Phytotoxicity was not observed at the highest rate of poultry manure (20 t/ha). Thus, the highest *Meloidogyne incognita* population suppression, growth and yield enhancement of okra was obtained with the highest spore densities of both species of *Trichoderma* in combination with amendment of poultry manure at 20 t/ha.

Conclusions and Recommendations

A comparative study between different rates of poultry manure and different spore load of *Trichoderma* species showed that increase in poultry manure rates and spore load of *Trichoderma* applied singly significantly reduced root galling, nematode density in soil per pot and nematode reproduction factor. Also, the combination of different rates of poultry manure with either species of *Trichoderma* at various spore loads significantly inhibited root galling and nematode population more than single application of each control agent.

Acknowledgement

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Novelty Statement

The study has brought to limelight the possibility of using indigenous isolates of *Trichoderma viride* and *Trichoderma harzianum* grown on a common agricultural produce (millet) in the management of *Meloidogyne incognita*.

Author's Contribution

Daniel Offiong Etim: Designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of manuscript.
Idorenyin Asukwo Udo and Etim Johnson Umana: Managed the analysis of the study.
Rosemary Anietie Bassey and Victoria Barrong Ogar: Managed the literature searches. All authors read and approved the final manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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