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Effects of *F. glycine*, plant materials and Organic matter onmalformation characteristics of two Soybean varieties (TGX1448-2E and TGX1485-ID) infected with Root-knot nematode (*MeloidogyneSpp.*) in green house, Micro-plot and field investigation.

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Abstract

Two-year green-house studies to evaluate the effects of Fusarium glycine, plant materials and organic matter in control of root-knot nematode (Meloidogyne spp.) using two Soyabean varieties(TGX1448-2E AND TGX1485-ID)were conducted in the National Root Crops Research Institute Umudike and College of Crop and Soil Science, Michael Okpara University of Agriculture, Umudike.In the green house experiment, two soybean cultivars were used. The treatments in Completely Randomized Design (CRD) and were replicated 3 times (2x10x3) to 60 large poly bags measuring 12.5cm diameter containing the sterilized soil was placed on greenhouse benches at a mean temperature of 27^{0}C. The treatments were as follows: Nematode alone, Nematode + poultry manure, Nematode + Fusarium glycine(F), Nematode + Dactyladenia barteri, Nematode + Dactyladenia barteri + Fusarium glycine, Nematode + Dactyladenia barteri+ Fusaruimglycine + Poultry manure(PM), Nematode + carbofuran, Control (no nematode no treatment), Dactyladenia barterialone and Fusarium glycine alone.In the green house, Soyabean variety TGX1485-ID had significantly (P≤0.05) higher number of eggs in roots (899) than those of TGX1448-2E variety (401). In the Mainplot trial, TGX1448-2E variety significantly (P≤0.05) produced higher Nematode larvae in soil (3134) than TGX1485-ID variety (1430). Poultry manure had highest (P≤0.05) number of galls (38.2) and number ofpods (47.4). Though, highest (P≤0.05)number of initial larvae was recorded on Fusarium glycine + PM.In conclusion, this study has revealed that apart from the chemical cabofuran which is not environmentally friendly because of toxicity, many other nematicides produced from biological agents (bionematicides) such as Dactyladenia barteri, Fusarium glycine and poultry manure in single mixtures and in combinations are cheap, readily available in abundance could serve as bionematicides in control of soyabean nematodes and reduce the problem of pollution and toxicity in the environment. These plant materials and organic matter are not only readily available but also cost effective and are therefore recommended to soyabean farmers as potential bionematicides in control of root-knot nematode (Meloidogyne Spp.) in Soyabean health and production.

Introduction: Root-knot nematodes, due to their high reproductive potential and wide host ranges are notoriously difficult to manage (Kalu, 2013). *Meloidogyne spp* requires 99.9% control in order to prevent the subsequent buildup of damaging population (Kalu, 2013). Some species of nematodes such as *M. incognita* is an important pest of crop ranging from vegetables, cereals, legumes to perennial crops (Sikoma and Fermandes, 2005). Plant parasite nematodes are microscopic round worms that are widely distributed and persist as soil plant pest for indefinite period (Obuezie and Ikpeze, 2012). Plant parasite nematodes are categorized into two groups based on their mode of feeding Ecto-parasite the root, and endo-parasite which undergo at least on stage of development inside the plant (Chen *et al*., 2012). Plant parasite nematodes are capable of causing direct severe damages to growing plants by making injury while the degree of such injury depends on the level of soil infestation, new races and prevailing environmental conditions such as drought, water logging and inadequate plant nutrition.

However, there is differential susceptibility of plants to root-knot infection. Wrather *et al.* (2003) showed that soil infested with *M.*

incognita and planted with tomatoes (*Lycopersicum esculentum*), Okra (*Abelmusclus esculentum*) and eggplant (*Solanum melongina*) had an increased nematode population at harvest of 69% for eggplant a poor host. The eggs are located on the root surface or inside galls (Moens *et al.*, 2009). Embroyogenesis results on a first stage juvenile that continues to develop inside the egg. When the juvenile has reached the second stage of development it is ready to hatch. Egg hatch depends on various environmental factors such as moisture, temperature and concentration of plant excretes. The aim of the study was therefore to determine theeffects of *F. glycine*, plant materials and Organic matter (either alone or in combination) onmalformation characteristics of two Soya-bean varieties (TGX1448-2E and TGX1485-ID) infected with Root-knot nematode (*MeloidogyneSpp*.) in green house, Micro-plot and field investigation.

Root-knot nematodes (*Meloiologyne spp*) cause varying degrees of stunting, sclerosis and in some cases early senescence, depending on the initial population density. Losses can often be related to the intensity of galling, which is also dependent on the initial population density (Sikora *et al*., 2005). Losses of 90% due to *M. incognita* have been reported from Florida, USA (Kinloch, 1974). The damage caused to soybean is also influenced by environmental factors, especially moisture, soil fertility status, soil compaction and soil Ph.

There are host plant resistance, cultural practices, biological control and nematicide application. This practice is in agreement with the global call to reduce agrochemical inputs into crop production (Holderness *et al*., 2000; Khan *et al*., 2002; Oyekanmi *et al*., 2008). Management of plant-parasitic nematodes using plant products and their derivatives is gaining importance in the light of increased awareness of environmental and human health hazards association with nematicidal chemicals.

Material and Methods: Experimental Site and Location: This study was carried out at green house of National Root Crops Research Institute Umudike, Abia state, Nigeria and Micro plots at Michael Okpara University of Agriculture Umudike Abia State Nigeria. National Root Crop Research Institute is within latitude, $05^0$29N and Longitude $07^0$33'E, 122m above level while Michael Okpara University of Agriculture, Umudike lies within latitude 05^0 291' and longitude $07^0$32'E at 120m above sea level. They located within the tropical rainforest ecological zone of south eastern Nigeria and the area is characterized by bimodal rainfall with one peak in July and second in September with annual rainfall of 2175mm and average humidity of 70-72% (NRCRI, 2012).

Experimental Design and Procedure: A two year experiments were carried out. The experiments were two-way factorial with two varieties of soybeans TGX1485-1D and TGX1448-2E in a Completely Randomized Design (CRD) for the green house and Micro plot experiments. The greenhouse experiment was carried out at the National Root Crops Research Institute Umudike, while the Microplot experiment was carried out in Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

Preparation of Plant Extracts: Ash of *Dactyladenia barteri*(Icheku) was collected. Three tons/hectare of *Dactyladenia barteri*ash was used (Campbell, 1990).

Poultry manure collection: Poultry manure was collected and dried and heated with big basin on the fire to reduce microbial component that was in it. Poultry manure was used at 10 tons/per hectare.

Extraction of Nematode Eggs: Nematode eggs were obtained from a culture of nematode infected roots of Indian Spinach (*Basella alba*). Called roots of *Basella alba* containing egg masses were cut into small pieces and placed in a container of 500ml capacity flask with 200ml of 0.15% chlorox (sodium hypochloride, NaOCI) solution shaken vigorously for 4mins (Hussey and Baker, 1973). This was done in order to digest the gelatinous matrix encasing the eggs. The solution was therefore poured through two nested sieves, 200 mesh (75um) and 500 mesh (25um). The eggs in the 500 mesh sieves, were washed free of NaOC1 solution with slow stream of cold tap water into a container previously marked to contain one litre. The cut roots in the original container were washed twice with water to obtain additional eggs. The number of eggs per 1ml suspension was estimated by counting four samples of 1ml each using Domncasters counting dish under a stereomicroscope and a working mean of egg/ml was estimated.

Isolation of Fungi (*Fusarium glycine*): *Fusarium* was isolated from infected soybean seeds, through the enumerated methods below. Infected seeds were sterilized with sterile distilled water. Then the seeds were transferred into sterile bale of filter paper to blot out the water droplets. Potato dextrose Agar (PDA) was prepared and allowed to solidity in a 2 cm petridish. The sterilized seeds were transferred into the prepared PDA plates with flamed forceps equidistantly. The plated plates were transferred

into an incubator at 25^0C for 3-5days. Plates were observed daily for any mycelia growth.

Sub culturing: The grown mycelia from the incubated plates was sub cultured (transfer) into a newly prepared potato Dextrose Agar plate with the aid of a sterile inoculating needle. The inoculation plates were transferred into an incubator at 25^0C for 3-5 days. The purified culture/microorganisms or fungi were transferred into slant dish for characterization.

Procedure for Characterized: The physical structures of the fungi like fluffy, texture, colour raised or not raised were observed. A wet-mount method (Fawole and Oso, 1988) was used to view the internal structure of the fungi under a compound microscope. The following structures were observed e.g. whether the fungi were septate or not, sporaniophores, spores, special growth features like rhizoid, was observed and compared with mycology atlas.

Inoculation with *Fusarium* (Fungi): A 4-5 days pure culture plate of *Fusarium* in a 9 cm petri-dish was used to wash 10mls of sterile distilled water. The sterile water was added to dilute the spore concentration. The spore used for inoculation was counted with a haemocytometer counting chamber for spore quantification before inoculating the plants. After inoculation a conductive environment was created by watering and covering with nylon bags for some hours to reduce evaporation of the fungi solution.

Soil Sterilization: Sandy-loam top soil rich in organic matter was taken in bulk. This was moistured and put in a drum. The soil drum, covered and heated until it reached a temperature of 80^0C using a soil thermometer and maintained at this temperature for 20-30 minutes. The soil was allowed to cool by spreading on a clean cement floor 48-60hours before use i.e. 2-4 days. After cooling, 10kg of the soil was separately put in 60 bags for trial, 60 bags for each plot of greenhouse experiment.

Experiment I: Green House Experiment: Experimental procedure: Two soybean cultivars were used. The treatments were in Completely Randomized Design (CRD) and replicated 3 times to 30 large poly bags measuring 12.5cm diameter containing the sterilized soil was placed on greenhouse benches at a mean temperature of 27^0C. They were placed in a distance to avoid contamination of the control plants. Four (4) soybean seeds (TGX 1485-1D, TGX 1448-2E) were sown in each pot at a depth of 2-3cm and was later thinned to one plant per pot. Three weeks (21 days) after germination the plants were inoculated close to their roots with 1000 eggs of freshly hatched eggs of *Meloidogyne incognita*. The treatment was applied on the same day of inoculation. *Dactyladenia barteri* ash were applied at the rate of 25 g each while *Fusarium glycine* and carbofuran were applied at the rate of 20 mls and 3.0 g a.i. respectively. Poultry manure was applied at the rate of 25 g, there were 10 treatments. The control was made up of untreated and uninnoculated plants. The treatments were as follows:

i. Nematode alone
ii. Nematode + poultry manure
iii. Nematode + *Fusarium glycine*
iv. Nematode + *Dactyladenia barteri*
v. Nematode + *Dactyladenia barteri*+ *Fusariumglycine*
vi. Nematode + *Dactyladenia barteri*+ *Fusaruim glycine* + poultry manure
vii. Nematode + carbofuran
viii. Control (no nematode no treatment)
ix. *Dactyladenia barteri*alone
x. Fusarium glycine alone

After treatment N.P.K fertilizer 15:15:15. Was applied at the rate of 100kg/ha (Ijoyah and Dzer, 2012) that is 0.4g per pot and was applied at four weeks after planting. The pots were kept free of weed by hand picking of weeds and watered. At three to four months (90 - 130 days) after planting the experiment was terminated and data collected included: Plant height (cm), number of leaves per plant, fresh root weight (g), dry root weight (g), fresh shoot weight (g) and dry shoots weight (g), number of pods, weight of pods (g) and number of nodules. Plant height was recorded by using measuring tape.

The number of leaves, pods, seeds and nodule per plant were counted, fresh and dry shoots weight, fresh and dry roots weight, weight of pods and dry weight of seeds where determined using an electronic weighing balance.

Experiment 2: Micro Plot Trial: Procedure: The micro plot experiment was carried out in Michael Okpara University of Agriculture Umudike. Thirty (30) large poly bags were filled measuring 12.5cm each with sterilized soil four soybean seeds (TGX 14-85-ID, TGX 1448-2E) were sown in the poly bags at a depth of 2-3cm

this was later thinned to one plant per pot. The pots were arranged in a CompleteRandomized Design (CRD) and were replicated 3 times for the Micro plot trial. Three weeks after planting the plants were inoculated close to their roots with 5000 eggs of freshly hatched eggs of *Meloidogyne incognita*. N.K.P. 15:15:15 fertilizers was applied at the rate of 100kg/ha (Ijoyah and Dzer, 2012) three weeks after planting. Treatments were applied on the same day of inoculation as Experiment 1. Each treatment was replicated three times. Hand picking of weeds were carried out when noticed. *Dactyladenia barteri*ash (*icheku*), was applied at 25 g each while spores of *Fusarium glycine* and *carbofuran* were applied at the rate of 20 mls and 30 g respectively and poultry manure was applied at the rate of 25 g. There were eight treatments, the actual and control untreated plots. The treatments were as in Experiment 1.

Data Collection: number of leaves per plant, fresh root weight (g), fresh shoot weight (g), dry shoot weight (g), dry root weight (g), number of pods, weight of pods (g), number of nodules, plant height (cm) at harvest, dry seed weight and number of seeds (g) were recorded.

Estimation of Nematode Population from soil: Nematodes were extracted from rhizospheric soil samples using the Pipan modification of the Bearman funnel method. Each soil sample from the sample bag was mixed thoroughly and 200mg placed into a plastic sieve and the tray lined with serviette paper. Enough water to soak the soil in the plastic sieve plates was gently added into the tray. The set up was then left for 24 hours before decanting the suspension into a beaker. A sample (2mls) of each suspension was drawn with a syringe after it has been properly mixed with stirrer into a watching glass and placed under a light microscope. Nematodes present were counted and recorded. This was repeated four times and average count was used to multiply the total volume of the suspension to get the estimated number of nematodes in the soil.

Estimation of Nematode Population from root: Nematode eggs in the roots were estimated by weighing out 2.0 g each of the root samples of the soybean plants infested with nematodes. Galled root samples were cut into small pieces and placed in a beaker of 500 ml, and thereafter processed as previously described in extraction of nematode eggs from soil.

Days to Flowering: This is carried out by calculating from the days after sowing until 50% of the plant have one or more flowers

Number of nodules: this was done at harvest.: This was done at harvest for the potted micro experiment by turning the pots upside down and carefully freeing the roots after they were washed carefully under the stream or tap, was mopped, dry and assessed under a microscope.

Fresh shoots weight: This was carried out both on the potted trials and Microplot. The fresh shoots of each treatment removed were weighed.

Fresh roots weight: this was carried out on the roots after removing the shoots. The weight of the fresh roots was recorded in gram.

Number of Leaves and Plants height:: This was done by measuring the height of each plant and number of leaves counted.

Dry Shoot/Dry Root Weight

Both the fresh shoots and fresh roots were taken to the oven and dried for eight hours at temperature of 100^0C the weight was then recorded in grams.

Number of pod: This is determined at harvest by counting the number of pods per plant for the potted trials and for the Microplot by counting the number of pods in each Pot.

Pod weight in Gramms (g)

This is done by weighting both the pods of the green house and that of the Microplot with a weighting balance with a precision of at least + 5g.

Pot/pot yield: For the Microplot and Greenhouse, dry seeds from each pot was weighed in grams.

Isolation of Fungi (*Fusarium glycine*): *Fusaruim glycine* was isolated from infected soybean seeds, though these methods, infected seeds were sterilized with sterile water. The seeds were transferred into sterile bale filtrate paper to blot out the water droplets Potato dextrose Agar (*PDA*) was prepared and allowed to be solidified in a 2cm petridish. The sterilized seeds were transferred into the prepared PDA plates with flamed forceps equidistantly. The plated plates were transferred into an incubator of 25^0C for 3-5 days and the plates were observed daily for any mycelia growth.

Sub Culturing: The grown mycelia from the incubated plate was sub-cultured (*transfer*) into some newly prepared potato Dextrose Agar plate with the aid of a sterilized inoculation needle. The inoculation plate was transferred into an incubator at 20^{0C} for 3-5days. The purified culture/microorganism or fungi was transferred into slant for characterization.

Procedure for Characterization: The physical structure of the fungi like fluffy, texture, colour raised or not raised was observed. A wet-mount method (Fawole and Oso, 1988) was used to view the internal structure of fungi under a compound microscope. The following structure was observed e.g. whether the fungi is septate or not, spurring spores, special growth features like

the rhizoid was observed and compound with mycology atlas.

Inoculation with *Fusariumglycine*: A 4-5days pure culture plate of *F. glycine* in a 9cm Petri dish was used to wash 10 of sterile distilled water. The sterile water was added to dilute the spore concentration. The spore used for inoculation was counted with a haemocytometer counting chamber for spore quantification before inoculating the plants. After inoculation, a conducive environment was created by watering and covering with ceylon bags for some hours to reduce evaporation of the fungi solution.

Preparation of Plant Extracts: Ash of *Dactyladenia barteri*(icheku) were collected and was thoroughly burnt. 3 tons/hectare of *Dactyladenia barteri*ash was used (Cambell, 1990).

Preparation of Extract

As stated in experiment (1)

Experimental Design and Statistical Analysis

Experimental Design

The experiment was appropriate in Completely Randomized Design (CRD) and replicated 3 times.

Statistical model:

$Y_{ij} = U + O_i + E_{ij}$

Where,

Y_{ij} = Individual observations of i^{th}organic amendments

U = Population mean

O_i= Effect of i^{th}organic amendments

E_{ij}= Random error, assumed to be independently, identically and

normaly distributed with zero mean and constant variance [iind $(0,\sigma 2)$].

Statistical Analysis : All data generated in the study were subjected to analysis of variance (ANOVA) using Genstat (3) 2007 and treatment means which showed significance were compared using Fisher's least significant difference (F-LSD) at 5% level of significance.

Results and Discussion: Results: Effects of *F. glycine*, plant materials and Organic matter (either alone or in combination) on No. of galls in roots, No. of eggs in roots and Nematode Larvae in soil on roots of two Soya-bean varieties infected with Root-knot nematode in green house and Micro-plot

Application of *Dactyladenia barteri*alone and Fusarium glycine alone significantly ($P \leq 0.05$) suppressed galling incidence and number of eggs in roots on Soyabean both in the green house and Mainplot trial (Table 4.6). Uninoculated soyabean plants had no gall while plants treated with Nematode + *Dactyladenia barteri*+ *Fusarium glycine*, Nematode + *Dactyladenia barteri* + *Fusaruim glycine* + poultry manure, Nematode + *Fusarium glycine*, Nematode + carbfuran and Nematode + poultry manure were slightly galled. Severe galling, number of eggs in roots and Nematode larvae in soil howeversignificantly ($P \leq 0.05$) occurred when Nematode alone was applied, followed by Nematode + poultry manure and Nematode + *Dactyladenia barteri*+ *Fusarium glycine* in both green house and Mainplot trial.

In green house and Mainplot trial, number of eggs produced was significantly ($P \leq 0.05$) reduced when No treatment was applied, and also when *Dactyladenia barteri*alone and *Fusarium glycine* alone were applied. Green house trial showed that soya bean variety TGX1485-IDsignificantly ($P \leq 0.05$) had higher number of eggs in roots (899) than those of TGX1448-2E variety (401).

In the Mainplot trial, TGX1448-2E variety significantly ($P \leq 0.05$) produced higher Nematode larvae in soil (3,134) than TGX1485-ID variety (1,430).

Table 1: Effects of *F. glycine*, plant materials and Organic matter (either alone or in combination) on No. of galls in roots, No. of eggs in roots and Nematode Larvae in soil on roots of two Soya-bean varieties infected with Root-knot nematode in green house

Treatments	No. of galls in roots	No. of eggs in roots	Nematode Larvae in soil
Control (no nematode, no treatment)	0.00	0.00	0.00
***Dactyladenia barteri*alone**	0.00	0.00	12
***Fusarium glycine* alone**	0.00	0.00	96
Nematode alone	17.17	1931	5667
Nematode + *Dactyladenia barteri*	0.50	112	259
Nematode + *Dactyladenia barteri+ Fusarium glycine*	4.50	1067	921
Nematode + *Dactyladenia barteri+ Fusaruim glycine* + poultry manure	4.42	908	2758
Nematode + *Fusarium glycine*	6.00	425	564
Nematode + carbofuran	4.08	825	867
Nematode + poultry manure	8.67	1232	2842
LSD (0.05)	**1922.4*** **	**1482.6*** **	**1689.7*** **
Varieties			
TGX1448-2E	4.53	401	1174
TGX1485-ID	4.53	899	1623
LSD (0.05)	**NS**	**328.0****	**NS**

*LSD = Least significant difference, * = significant, NS = not significant*

Table 2: Effects of *F. glycine*, plant materials and Organic matter (either alone or in combination) on No. of galls in roots, No. of eggs in roots and Nematode Larvae in soil on roots of two Soya-bean varieties infected with Root-knot nematode in Micro plot

Treatments	No. of galls in roots	No. of eggs in roots	Nematode Larvae in soil
Control (no nematode, no treatment)	0.00	0.00	0.00
***Dactyladenia barteri*alone**	0.00	0.00	0.00
***Fusarium glycine*alone**	0.20	0.00	0.00
Nematode alone	46.30	6150	8817
Nematode + *Dactyladenia barteri*	1.90	42	379
Nematode + *Dactyladenia barteri*+ *Fusarium glycine*	8.80	821	1358
Nematode + *Dactyladenia barteri* + *Fusaruim glycine* + poultry manure	17.80	1934	4776
Nematode + *Fusarium glycine*	4.70	1376	842
Nematode + carbofuran	10.2	877	1012
Nematode + poultry manure	12.90	1559	5633
LSD (0.05)	**11.78*** **	**2216.1*** **	**1854.4*** **
Varieties			
TGX1448-2E	11.4	1216	3134
TGX1485-ID	9.10	1336	1430
LSD (0.05)	**NS**	**NS**	**829.3*** **

*LSD = Least significant difference, * = significant, NS = not significant*

Effect of *Fusarium glycine*, plant materials and Organic matter (either alone or in combination) on mean number of leaves, number of noddles, number of galls, number of number of eggs, number of initial larvae, number of mid larvae, number of final larvae, number ofpod and number of Seed of Soya-bean infected with Root-knot nematode in the field trial.

Table 3 shows the effectsof *F. glycine*, plant materials and Organic matter (either alone or in combination) on mean number of leaves number of noddles, number of galls, number of number of eggs, number of initial larvae, number of mid larvae, number of final larvae, number ofpod and number of Seed of Soya-bean infected with Root-knot nematode in the field trial. The field trial experiment showed that Control (No nematode) and application of Poultry manure (PM) significantly (P≤0.05) influenced the number of leaves number of noddles, number of galls, number of number of eggs, number of initial larvae, number of mid larvae, number of final larvae, number ofpod and number of Seed with control having highest number of leaves (27.42), number of eggs (6200), number of mid larvae (2,500) and number of final larvae (2,275). Whereas, Poultry manure had highest (P≤0.05) number of gall (38.2), number ofpods (47.4) and number of seed (185.7). Though, highest (P≤0.05)number of initial larvae was recorded on *Fusarium glycine* + PM.

However, TGX1485-ID was significantly (P≤0.05) higher in number of noddles (9.0), number of galls (20.1), number ofpods (32.5) and number of seed (126.4) than TGX1448-2E while highest (P≤0.05) number of mid larvae and number of final larvae were recorded in the variety, TGX1448-2E. Also, there was no significant ($p \geq 0.05$) difference between *F. glycine*, plant materials and Organic matter and their combinations on number of leaves, number of eggs and number of initial larvae in soil of the two Soya-bean varieties.

Table 3: Effect of *Fusarium glycine*, plant materials and Organic matter (either alone or in combination) on mean number of leaves, noddles, galls, eggs, initial larvae, mid larvae, final larvae in soil, number ofpod and number of Seed of Soya-bean infected with Root-knot nematode in the Field Trial.

Treatments	No. of Noddle	No of leaves	No of Galls	No of eggs	No of Initial Larvae	No of Mid Larvae	No of Final Larvae	No of Pods	No of Seed
Control (no nematode, no treatmt)	2.42	27.42	30.9	6200	1775	2500	2275	10.1	40.2
***Dactyladenia barteri*alone**	6.92	18.75	0.9	212	1033	1275	300	27.1	108.3
Dactyladenia barteri+ Fusarium glycine	6.17	11.00	4.1	1017	1442	1208	442	20.7	82.3
***Dactyladenia barteri+ Fusarium glycine* + PM**	12.67	13.42	4.2	442	1208	1092	408	17.0	67.3
***Fusarium glycine* alone**	5.58	11.92	2.0	233	1433	1175	700	32.2	118.2
***Fusarium glycine* + PM**	8.25	10.92	14.2	975	2067	1783	1367	28.1	128.0
Nematicide	5.92	23.50	2.8	292	1683	958	408	24.5	98.0
PM	7.25	18.33	38.2	1933	1650	1658	650	47.4	185.7
L.S.D(0.05)	NS	**2.084***	**16.73***	**2421.0***	**619.9***	**669.3***	**643.1***	**20.02***	**80.69***
Varieties									
TGX1448-2E	4.79	16.92	4.1	1107	1479	1731	867	19.3	80.6
TGX1485-ID	9.00	16.83	20.1	1719	1594	1181	771	32.5	126.4
LSD (0.05)	3.381*	**NS**	**8.36***	**NS**	**NS**	**334.7****	**5.45****	**10.01***	**40.35***

*LSD = Least significant difference * = significant, ns = not significant.*

PM = Poultry manure

Discussion: The amount of damage caused by plant parasitic nematodes is related to many variables, including the nematode species, the size of the nematode population, the susceptibility of the host plant and various environmental factors such as temperature, duration of the growing season, availability of water and nutrients to the plant and the presence of other organisms contributing to the total damage inflicted upon the crop (Back *et al*., 2002). There was influence on the number of leaves and plant height with Nematode + poultry manure and Nematode + carbofuran treatments. This is in agreement with Joshi and Patel (1995) who reported that application of poultry manure showed improved growth of groundnut crops and reduced nematode population. Report has showed that some *Fusariumspp* and *Trichoderma* isolates enhanced plant growth and reduced root knot damage (Meyer *et al.,* 2001).

Meloidogyne spp. requires 99.9% control in order to prevent the subsequent buildup of damaging population (Kalu, 2013). From the results obtained for *Fusarium glycine* in combination with the organic materials showed significant effect on checking the nematodes and this proves that the use of synthetic nematicide is the most producing and most affect practical means of combating the menace of the plant parasitic nematode (Ononuju and Fawole, 2000) This is attributable to the fact that root not nematodes attack the roots of soyabean. This result is inline with the report that endoparasitic nematodes such as *Meloidogyne* and *Heterodera* species are more disruptive to their host's roots (Back *et al*., 2002). As nematodes steal nutrients from the roots, the plants are weakened and do not grow well. Subsequently, plants may be more vulnerable to attack by other stresses such as insects, diseases and drought. Part of the damage done by plant parasitic nematodes to soybean includes the damage to the root system, which causes sloughing off of the root and severe necrosis. Root pruning as well as proliferation of lateral roots may occur, and a reduction of the grain yield is normally seen (Lowe, 1992).

Uninoculated soyabean plants had no gall while plants treated with Nematode + *Dactyladenia barteri*+ *Fusarium glycine*, Nematode + *Dactyladenia barteri*+ *Fusaruim glycine* + poultry manure, Nematode + *Fusarium glycine*, Nematode + Carbofuran and Nematode + poultry manure were slightly galled.

This observation agreed with that of Agu (2008) reported significant control of root-gall disease of pineapple with poultry manure. Poultry manure contains a significant amount of nitrogen, the majority of which is in the form of uric acid that can be easily converted to ammonia, which is lethal to plant-parasitic nematodes. The *Fusarium glycine* combination effects is similar to the. isolates of *Trichoderma fusarium* against *Meloidogyn*e *spp.*in tomatoes which provided significant inhibition of nematode reproduction, suppression of root galling and an increase of tomato yield (Affokpon *et al*., 2011).

The matured females of root not nematode lay eggs in the roots. A female lays several hundred eggs, which are deposited in a gelatinous matrix through a rupture in the galled surface. These give rise to the first-stage juveniles, which are enclosed within the egg; second-stage juveniles emerge after about 10 days (Oyekanmi and Fawole, 2010). These chemicals either affected the embryonic development or kill the eggs or even dissolved the egg masses. It has been reported (Adegbite 2003; Hackney and Dickson, 1998) that extracts contained alkaloids, flavonoids, saponins, amides including benzamide and ketones that singly and in combination inhibited hatching.

Severe galling, number of eggs in roots and Nematode larvae in soil howeversignificantly ($P \leq 0.05$) occurred when Nematode alone was applied, followed by Nematode + poultry manure and Nematode + *Dactyladenia barteri* + *Fusarium glycine* in both green house and Mainplot trial indicating that number of eggs in roots and Nematode larvae in soil is dependent on the level of maturity of the soyabean since the number of nematodes in the soil reaches a maximum at soybean maturity. The population declines slowly through the winter and then precipitously as soil temperatures increase in the spring. The rapid decrease in the number of infective nematodes in the soil can occur several weeks before soybean planting due to factors such as increased activity of the nematodes and depletion of their food reserves, along with an increase in the predatory and parasitic activities of the soil arthropod and microbial communities, which are detrimental to the survival of the nematodes. At planting time, the number of nematodes in the soil is at its lowest, usually <10% of that at soybean maturity (Sinclair and Backman, 1989).

Root knot nematodes cause histopathological changes in root tissues of pointed gourd resulting the formation of giant cells and galls in the root system. These abnormalities or malformations can upset the normal physiological activities of vascular tissue of the root system and causes wilting, stunting, leaf chlorosis and poor growth of plants (Wrather *et al.* (2003).

Conclusion: The study has revealed that apart from the chemical cabofuran which is not environmentally friendly because of toxicity, many other nematicides produced from biological agents (bionematicides) such as *Dactyladenia barteri, Fusarium glycine* and poultry manure in single mixtures and in combinations are cheap, readily available in abundance could serve as bionematicides in control of soyabean nematodes and reduce the problem of pollution and toxicity in the environment. These plant materials and organic matter are not only readily available but also cost effective and are therefore recommended to soyabean farmers as potential bio-nematicides in control of root-knot nematode (*Meloidogyne Spp.*) in soyabean production. Moreover, it was observed that root-knot nematode (*Meloidogyne Spp.*) alone affected Soyabean varieties (TGX1485-ID and TGX1448-2E).

The organic amendments (Nematode + poultry manure and Nematode + *Dactyladenia barteri*+ *Fusarium glycine)* were found to have nematicidal properties. This finding is important from the point of view of controlling root-knot nematodes affecting edible soybean without the use of nematicides in view of the environmental pollution likely to cause. The future looks bright for identifying new classes of pesticides from natural plants to replace the synthetic dangerous and expensive chemicals used at present. The cooperation of nematologists, breeders, chemists, ecologists and others in the field of agriculture is necessary to achieve maximum progress in this important field of research.

Further research for developments in soybean nematode management and information sharing should be encouraged and supported. This effort, if consolidated and well-funded, will give birth to promising eco-friendly strategies in sustainable soya bean production in particular and agriculture in general.

Furthermore, there is need to identify the mode of action of these potential bio-pesticides and to develop new stable and low cost nematicidal and nematostatic formulations from plants sources and organic materials.

In the field trial, poultry manure formulation showed high biopesticidal and nematicidal importance and therefore could be used for the control of root-knot nematode (*Meloidogyne Spp.*).

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