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Effects of Combination *F. Glycine*, Plant Materials and Organic Matter on Mean Growth and Yield Parameters of Two Soybean Varieties Infected with Root-Knot Nematode (*Meloidogyne Spp.*) in Green House and Micro-Plot.

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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 27^0C. The treatments were as follows: Nematode alone, Nematode + poultry manure, Nematode + Fusarium glycine (F), Nematode + Dactyladenia barteri (Db), Nematode + Dactyladenia barteri (Icheku) + Fusarium glycine, Nematode + Dactyladenia barteri + Fusaruim glycine + Poultry manure (PM), Nematode + carbofuran, Control (no nematode no treatment), Dactyladenia barteri alone and Fusarium glycine alone. The green house experiment showed that Control and application of Nematode + poultry manure significantly (P≤0.05) influenced the number of leaves and plant height with Nematode + poultry manure and Nematode + carbofuran treatments having average number of 20.08 leaves and 63.43 cm respectively. However, TGX1485-ID was significantly (P≤0.05) higher in number of leaves than TGX1448-2E. Whereas in main-plot, Control and application of Nematode + poultry manure significantly (P≤0.05) influenced the number of leaves while Nematode + carbofuran treatment influenced (P≤0.05) higher plant height at average of 64.34 cm respectively. However, TGX1485-ID was significantly (P≤0.05) higher in number of leaves than TGX1448-2E in Green house. Nematode alone and Nematode + Dactyladenia barteri + Fusaruim glycine + poultry manure significantly (P≤0.05) influenced the number of nodules (2.750) and fresh shoot weight (13.50g) in greenhouse. However, TGX1448-2E variety was significantly (P≤0.05) higher in number of nodules than in variety TGX1485-ID. TGX1485-ID variety had significantly (P≤0.05) higher number of nodules in Micro plot trial than variety TGX1448-2E. F. glycine significantly (P≤0.05) affected other parameters apart from fresh shoot weight of the green house experiment. TGX1485-ID significantly (P≤0.05) higher in number of pods and dry weight of pods than TGX1448-2E in Micro plot trial while TGX1448-2E variety showcased heavier dry weight of pods in the green house. In conclusion, in negligence of 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 bionamaticieds in control of root-knot nematode (Meloidogyne Spp.) in Soyabean production.

Introduction: Root-knot nematodes (*Meloidogyne species*) are very important pests of soybean. Species include *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica*, which limit soybean grain yield, symbiotic nitrogen fixation (SNF) and growth (Sikora *et al*., 2005). Soybean is of great economic importance and as late as 1940; the area harvested for forage was equal to that harvested for grains (Geofery, 2007). Soybeans are today of major cash crop in United States, second only to corn in financial return to the farmer.Ogundipe 2010 showed that a given area of land planted with soybean can produce much more protein than the same land put to use for any other conventional farming operation.As reported by Kalu (2013), the root-knot nematodes, due to their high reproductive potential and wide host ranges are notoriously difficult to manage. *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). The employment of various sources of organic materials has been promoted as one of the principal sustainable management options for improving soil quality and productivity (Widmer *et al*., 2002). Organic amendments such as green manure, crop residues, cow dung and poultry manure used to improve soil fertility, have also been found to control root diseases including nematodes (Poswal and Akpal, 1991). The application of organic amendment to the soil as an alternative strategy for the management of plant-parasitic nematodes have been proved to substantially increase soil health (Neher, 2001), environmental wellness (Adegbite and Adesiyan, 2005) and sustainable crop production with no documented negative effect of organic soil amendment on non-target organisms (Agyarko and Asante, 2005). Oka *et al*. (2000) indicated that organic addition have constantly produced beneficial effects on soil nutrients, soil physical conditions, and soil biological activities thereby improving the health of plants and reducing populations of plant-parasitic nematodes. Poultry Manure significantly reduced both root galling and nematode population with 4 t/ha significantly increasing yield characters of carrot (Kankam *et al*., 2014).

Adegbite and Adesiyan (2005) reported that the indiscriminate use of synthetic nematicides for controlling nematodes gives rise to phytotoxicity, environmental pollution and nematode resistance. Ononuju (1999) reported that then high cost, along with consideration of safety for the farmers and need for agricultural sustainability, have led many farmers, agriculturists and others to reconsider the use of chemical to control agricultural pests since these chemical affect the environment adversely. Majority of food crops are damaged by at least one species of nematodes and the economic consequences of nematodes infestation are many, reducing crop quantity and yield (Agrios, 2005*).*

Also report from Meyer *et al.* (2001), showed that some *Fusarium spp* and *Trichoderma* isolates enhanced plant growth and reduced root knot damage. The approaches of the using of bio control for the management of nematode has its constraints. Soybean (*Glycine max* (L.) Merril) is the important and well-recognised grain legume, vegetable oil and protein crop in many countries of the world (Ahmed *et al.*, 1996). Soybean has occupied the top position in terms of oil source in the world.

The use of synthetic nematodes has been found to be the most effective method of controlling plant parasitic nematodes (Ononuju and Fawole, 2000).

These 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). The concern over indiscriminate use of chemicals in the control of pests has led to the sourcing of alternatives that are effective, ecologically safe and economical. Such alternatives include the use of botanicals derived from very cheap and renewable sources, especially tropical plants (Fatoki, 2001 and Adegbite and Adesiyan, 2005).

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 (2013-2014) 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 9cm petric-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 *(Icheku*) 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 uninoculated 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* + *Fusarium glycine*
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), 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 Complete Randomized 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 25g. 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 (Hopper, 1969). 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: According to Barnette and Hunter (1987), 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 *Fusarium glycine* : 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: Effect of *Fusarium glycine*, plant materials and Organic matter (either alone or in combination) on mean number of leaves and plant height of Soya-bean infected with Root-knot nematode in green house and Micro-plot.

Table 1 shows the effects of *F. glycine*, plant materials and Organic matter (either alone or in combination) on mean number of leaves and plant height of Soya-bean infected with Root-knot nematode in green house and Micro-plot.

The green house experiment showed that Control and application of Nematode + poultry manure significantly ($P \leq 0.05$) influenced the number of leaves and plant height with Nematode + poultry manure and Nematode + carbofuran treatments having average of 20.08 leaves and 63.43 cm respectively. However, TGX1485-ID was significantly ($P \leq 0.05$) higher in number of leaves than TGX1448-2E. Lower number of leaves however occurred when nematode alone was applied.

Whereas in main-plot, Control and application of Nematode + poultry manure significantly ($P \leq 0.05$) influenced the number of leaves while Nematode + carbofuran treatment influenced ($P \leq 0.05$) higher plant height at average of 64.34 cm respectively. However, TGX1485-ID was significantly ($P \leq 0.05$) higher in

number of leaves than TGX1448-2E in Green house. Lower number of leaves however occurred when nematode alone was applied.

Table 1: Effects of *F. glycine*, plant materials and Organic matter (either alone or in combination) on mean number of leaves and plant height (cm) on roots of two Soya-bean varieties infected with Root-knot nematode in green house and Micro-plot

Green house	Micro-plot trial			
Treatments	**Number of leaves**	**Plant height (cm)**	**Number of leaves**	**Plant height (cm)**
Control (no nematode, no treatment)	21.25	50.82	22.83	52.76
***Dactyladenia barteri* alone**	11.58	42.01	13.67	40.67
***Fusarium glycine* alone**	10.75	41.73	11.25	38.79
Nematode alone	8.92	34.04	8.33	32.72
Nematode + *Dactyladenia barteri*	17.42	55.06	17.83	52.40
Nematode + *Dactyladenia barteri* + *Fusarium glycine*	17.92	41.82	10.58	40.08
Nematode + *Dactyladenia barteri* + *Fusaruim glycine* + poultry manure	10.33	39.45	10.00	40.00
Nematode + *Fusarium glycine*	13.58	47.73	13.42	44.19
Nematode + carbofuran	17.25	63.43	19.50	64.34
Nematode + poultry manure	20.08	59.04	20.75	60.95
LSD (0.05)	**2.938*** **	**2.938*** **	**1.184*** **	**3.293*** **
Varieties				
TGX1448-2E	13.62	46.74	14.30	45.96
TGX1485-ID	14.80	48.28	15.33	47.42
LSD (0.05)	**1.007***	**1.314***	**0.529*** **	**NS**

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

Effects of *F. glycine*, plant materials and Organic matter (either alone or in combination) on number of nodules, fresh shoot weight (g) and dry shoot weight (g) on roots of two Soya-bean varieties infected with Root-knot nematode in green house and Micro-plot. The green house experiment showed that two treatments: Nematode alone and Nematode + *Dactyladenia barteri* + *Fusaruim glycine* + poultry manure significantly ($P \leq 0.05$) influenced the number of nodules (2.750) and fresh shoot weight (13.50g) in the green house experiment. However, TGX1448-2E variety was significantly ($P \leq 0.05$) higher in number of nodules than in variety TGX1485-ID. On the other hand, TGX1485-ID variety had significantly ($P \leq 0.05$) higher number of nodules in Micro plot trial than variety TGX1448-2E. The fresh shoot weight and dry shoot weight on roots of the two Soya-bean varieties infected with Root-knot nematode in green house and Micro-plot were not significantly ($p \geq 0.05$) different.

Table 2: Effects of *F. glycine*, plant materials and Organic matter (either alone or in combination) on number of nodules, fresh shoot weight (g) and dry shoot weight (g) on roots of two Soya-bean varieties infected with Root-knot nematode in green house and Micro-plot

Green house			Micro-plot trial			
Treatments	Number of nodules	fresh shoot weight (g)	Dry shoot weight (g)	Number of nodules	fresh shoot weight (g)	dry shoot weight (g)
Control (no nematode, no treatment)	2.000	12.00	21.21	8.33	21.54	16.48
***Dactyladenia barteri* alone**	2.250	0.00	21.41	11.08	20.76	14.13
***Fusarium glycine* alone**	1.250	0.00	19.50	3.83	19.46	13.51
Nematode alone	2.750	13.50	13.32	0.92	13.98	8.92
Nematode + *Dactyladenia barteri*	2.167	0.67	18.77	5.92	19.02	14.09
Nematode + *Dactyladenia barteri* + fusarium glycine	2.167	4.97	22.54	3.42	21.53	15.97
Nematode + *Dactyladenia barteri* + Fusaruim glycine + poultry manure	2.750	2.83	20.68	10.33	20.73	14.88
Nematode + *Fusarium glycine*	1.833	6.00	23.40	2.58	20.38	14.72
Nematode + carbofuran	1.667	4.00	18.75	1.00	21.43	15.57
Nematode + poultry manure	2.250	7.17	21.12	1.50	21.12	15.68
LSD (0.05)	**0.8588***	**4.402*****	**NS**	**2.422*****	**4.442***	**4.005***
Varieties						
TGX1448-2E	2.317	3.80	14.20	4.25	20.09	14.20
TGX1485-ID	1.900	3.87	14.50	5.53	19.90	14.59
LSD (0.05)	**0.3841***	**NS**	**NS**	**1.083*****	**NS**	**NS**

*LSD = Least significant difference; * = significant; NS = not significant.*

Effects of *F. glycine*, plant materials and Organic matter (either alone or in combination) on fresh root weight (g) and dry root weight (g) on roots of two Soya-bean varieties infected with Root-knot nematode in green house and Micro-plot. Nematode alone significantly (P≤0.05) affected fresh root weight whereas Nematode + poultry manure and dry root weight in green house respectively.*F. glycine* significantly (P≤0.05) affected other parameters apart from fresh shoot weight of the green house experiment. There were no significantly ($p \geq 0.05$) difference in fresh root weight and dry root weight of two Soya-bean varieties infected with Root-knot nematode in Micro-plot trial. However, TGX1448-2E was significantly (P≤0.05) higher in dry root weight than TGX1485-ID varieties in green house, though not significantly ($p \geq 0.05$) different in fresh root weight (green house), and fresh root weight and dry root weight in Micro-plot trial (Table 3).

Table 3:Effects of *F. glycine*, plant materials and Organic matter (either alone or in combination) on fresh root weight (g) and dry root weight (g) on roots of two Soya-bean varieties infected with Root-knot nematode in green house and Micro-plot

Green house		Micro-plot trial	
Treatments	fresh root weight (g)	dry root weight (g)	fresh root weight (g)	dry root weight (g)
Control (no nematode, no treatment)	12.82	5.11	0.725	0.517
***Dactyladenia barteri* alone**	10.59	3.08	0.567	0.492

***Fusarium glycine* alone**	10.43	2.99	0.567	0.450
Nematode alone	13.12	4.11	0.492	0.392
Nematode + *Dactyladenia barteri*	11.84	4.30	0.542	0.458
Nematode + *Dactyladenia barteri* + fusarium glycine	10.99	3.29	0.575	0.475
Nematode + *Dactyladenia barteri* + *Fusaruim glycine* + poultry manure	11.16	3.64	0.942	0.775
Nematode + *Fusarium glycine*	10.97	3.58	0.483	0.425
Nematode + carbofuran	11.08	3.73	0.783	0.675
Nematode + poultry manure	12.77	4.84	0.508	0.417
LSD (0.05)	**1.354*** **	**0.932*** **	**NS**	**NS**
Varieties				
TGX1448-2E	11.32	4.23	0.620	0.515
TGX1485-ID	1.83	3.51	0.617	0.500
LSD (0.05)	**NS**	**0.416*** **	**NS**	**NS**

*LSD = Least significant difference: * = significant; NS = not significant.*

Effects of *F. glycine*, plant materials and Organic matter (either alone or in combination) on number of pods and dry weight of pods (g) on roots of two Soya-bean varieties infected with Root-knot nematode in green house and Micro-plot

The green house experiment showed that *Dactyladenia barteri* alone and Nematode + *Dactyladenia barteri* significantly ($P \leq 0.05$) influenced the number of pods (15.50) and dry weight of pods (17.38 g) while Micro-plot trial showed significant ($P \leq 0.05$) difference in number of pods (12.75) and dry weight of pods (20.96 g) as affected by *Dactyladenia barteri* alone as presented in (Table 4). Moreso, TGX1485-ID significantly ($P \leq 0.05$) higher in number of pods and dry weight of pods than TGX1448-2E in Micro plot trial while TGX1448-2E variety showcased heavier dry weight of pods in the green house. Lower number of pods and heavier dry weight of pods however, occurred when combination of Nematode + *Dactyladenia barteri* + *Fusaruim glycine* + poultry manure was applied. However, there was no significantly ($p \geq 0.05$) varietal difference in number of pods in the green house experiment as shown in Table 4.

Table 4: Effects of *F. glycine*, plant materials and Organic matter (either alone or in combination) on number of pods and dry weight of pods (g) on roots of two Soya-bean varieties infected with Root-knot nematode in green house and Micro-plot

Green house		Micro-plot trial			
Treatments		**number of pods**	**dry weight of pods (g)**	**number of pods**	**dry weight of pods (g)**
Control (no nematode, no treatment)		12.00	13.71	14.17	20.44
***Dactyladenia barteri* alone**		15.50	15.72	12.75	20.96
***Fusarium glycine* alone**		8.92	10.21	9.50	11.43
Nematode alone		6.17	6.07	6.50	3.67
Nematode + *Dactyladenia barteri*		15.25	17.38	11.75	17.89
Nematode + *Dactyladenia barteri* + *Fusarium glycine*		11.00	13.80	8.33	13.88
Nematode + *Dactyladenia barteri* + *Fusaruim glycine* + poultry manure		9.08	13.09	8.17	11.64
Nematode + *Fusarium glycine*		11.67	14.72	8.25	9.49
Nematode + carbofuran		9.17	9.36	12.33	12.15
Nematode + poultry manure		12.67	14.25	9.00	15.18
LSD (0.05)		**3.261*** **	**5.006***	**2.790*** **	**7.411*** **

Varieties				
TGX1448-2E	11.57	14.18	7.17	11.79
TGX1485-ID	10.72	11.33	12.98	15.55
LSD (0.05)	**NS**	**1.830****	**1.248****	**3.315***

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

Effects of *F. glycine*, plant materials and Organic matter (either alone or in combination) on number of seeds and dry weight of seeds (g) on roots of two Soya-bean varieties infected with Root-knot nematode in green house and Micro-plot

Effect of treatments on number of seeds and dry weight of seeds are presented in Table 5. In the green house, there were significant (P≤0.05) differences in number of seeds and dry weight of seeds of plants. *Dactyladenia barteri* alone and Nematode + *Dactyladenia barteri* had the highest (P≤0.05) number of seeds and heaviest dry weight of seeds respectively. TGX1448-2E had significantly (P≤0.05) heavier dry weight of seeds than TGX1485-ID variety in green house experiment. The two soya bean varieties did not differ significantly ($p \geq 0.05$) in number of seeds.However in the Microplot, significantly (P≤0.05) differences were observed on the number of seeds and dry weight of seeds. Uninnoculated plants produced the highest number of seeds (54.80), followed by plants treated with *Dactyladenia barteri* alone. For dry weight of seeds, plants that received *Dactyladenia barteri* alone recorded the heaviest dry weight of seeds (19.57 g), and differed significantly (P≤0.05) from all other treatments except control.

TGX1485-ID had significantly (P≤0.05) higher number (51.4) and heavier dry weight (13.87 g) of seeds than TGX1448-2E (Table 5).Table 5: Effects of *F. glycine*, plant materials and Organic matter (either alone or in combination) on number of seeds and dry weight of seeds (g) on roots of two Soya-bean varieties infected with Root-knot nematode in green house and Micro-plot

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

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. Report has showed that some *Fusarium spp* and *Trichoderma* isolates enhanced plant growth and reduced root knot damage (Meyer *et al.*, 2001).

The major treatments Nematode + poultry manure influenced the number of leaves and plant height of TGX1485-ID and TGX1448-2E varieties of soya bean which may be attributed to the presence of nematicidal substanaces like Nitrogen in the organic matters substances suggesting that these poultry manure (agrowaste) have the potential of being used for reducing nematode in soyabean production by farmers in Nigeria. Also, Nematode + carbofuran influenced the number of leaves and plant height of TGX1485-ID and TGX1448-2E varieties of soya bean both in green house experiment and Mainplot trial, which may be attributed to the fact that synthetic nematicide is the most producing and most effective practical means of combating the menace of the plant parasitic nematode (Ononuju and Fawole, 2000).

The yield in terms of number of nodules was highest with TGX1448-2E variety was significantly higher in number of nodules than in variety TGX1485-ID. On the other hand, TGX1485-ID variety had significantly higher number of nodules in Micro plot trial than variety TGX1448-2E as treated with Nematode alone and Nematode + *Dactyladenia barteri* + *Fusaruim glycine* + poultry manure indicating that the treatments could be used in the control of root knot nematodes which contributes to retardation of seedling growth, plant damage, sudden death and concomitant yield losses (Orisajo *et al.*, 2008). Infection by *Meloidogyne* species can also lead to reduced nodulation by nitrogen fixing bacteria. The fresh shoot weight and dry shoot weight on roots of the two Soya-bean varieties infected with Root-knot nematode in green house and micro-plot were similar. Poultry manure has been observed to decrease nematode population and increase plant height, shoot and root weight (Patel and Patel, 1992). *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). On the fresh root weight and dry root weight of the roots, very succulent soybean tissue in the soil,

especially the roots, is subject to attack by root-knot nematodes (Oyekanmi and Fawole, 2010). The result on the fresh root weight and dry root weight of the roots of TGX1485-ID and TGX1448-2E varieties of soya bean were affected by Nematode alone whereas Nematode + poultry manure and dry root weight in green house respectively. This could be attributable to the fact that root not nematodes attack the roots of soyabean. This result is in line 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.

There were no significantly difference in fresh root weight and dry root weight of two Soya-bean varieties infected with Root-knot nematode in Micro-plot trial indicating that these plant materials and Organic matter and or their combinations could be used to produce of Soya bean (TGX1485-ID and TGX1448-2E*).* The variations may be due to varying nutrients in the plant materials and Organic matter and or their combinations and different soya bean varieties used.

Similarly, infection due to *Meloidogyne* causes wounds on soybean root while feeding. These wounds provide entry points for secondary pathogenic infection. Thus, the plant becomes susceptible to root rotting organisms. *M. incognita* causes significant yield loss of soybean (Weaver *et al.*, 1988, Robbins *et al.*, 1990) by as much as 90% for susceptible soybean cultivars (Kinloch, 1974). Annual soybean yield losses to this nematode still exceed 99,600 metric tons in the United States of America (Wrather, 2003).

The study on the number of pods and dry weight of pods produced by TGX1485-ID and TGX1448-2E in relation to *F. glycine*, plant materials and Organic matter and or their combinations indicated that the green house experiment showed that *Dactyladenia barteri* alone and Nematode + *Dactyladenia barteri* significantly influenced the number of pods (15.50) and dry weight of pods (17.38 g) while Micro-plot trial showed significant difference in number of pods (12.75) and dry weight of pods (20.96 g) as affected by *Dactyladenia barteri* alone.

However, the green house experiment, showed significant effects on number of seeds and dry weight of seeds of the soya bean plants. *Dactyladenia barteri* alone and Nematode + *Dactyladenia barteri* had the highest number of seeds and heaviest dry weight of seeds respectively. The two soya bean varieties did not differ significantly in number of seeds.In the Microplot, uninnoculated soya bean plants produced the highest number of seeds (54.80), followed by plants treated with *Dactyladenia barteri* alone. For dry weight of seeds, plants that received *Dactyladenia barteri* alone recorded the heaviest dry weight of seeds (19.57 g) which implies that *Dactyladenia barteri* could be used reduced nematode population significantly and improve yield. Because it has been observed that root-knot nematodes, *Meloidogyne* species limits soybean grain yield (number of seeds), symbiotic nitrogen fixation (SNF) and growth (Sikora *et al*., 2005; Oyekanmi *et al*., 2007; Coyne and Oyekanmi, 2007).

Conclusion: 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 bionamaticieds 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).

Fusarium glycine on plant materials and organic matter and in combination exhibited superiority in control of root-knot nematode (*Meloidogyne Spp*.) in Soyabean cvariety (TGX1448-2E) in terms of yield and yield attributes(number of nodules dry root weight, dry weight of pods, dry weight of seeds) in Green house experiment than in TGX1485-ID soyabean variety. Whereas, in microplot experiment, TGX1485-ID exhibited superiority in control of root-knot nematode (*Meloidogyne Spp*.) in Soyabean variety (TGX1485-ID) in terms of yield and yield attributes (number of leaves, number of nodules, number of pods and dry weight of pods number and dry weight of seeds).

Finally, the results show that *Fusarium glycine* on plant extracts and organic matter such as *Dactyladenia barteri*, poultry manure and their combinations can be used as safe alternative nematicide for root- knot nematode control in the two soybean varieties, especially since carbofuran is a regulated pesticide due to its high toxicity. As root-knot nematode density in

the field increases, this prophylactic (preventive) management measures are required to avoid yield loss. This practice is in agreement with the global call to reduce agrochemical inputs into crop production. It has been concluded from present research that certain plant extracts are a source of cheap and effective nematicides of root knot nematodes. The root extracts of Neem, Siam weed, Lemon grass and Castor bean 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.

Recommendation: Based on the findings in this study, the following important recommendations are made. They include:Combination effect of *Fusarium glycine* and poultry manure could be used for the control of *Meloidogyne Spp.* on two soybeans varieties; Bio-nematicides from plant extracts and organic matter are relatively economical and cost effective because they can be gathered by soyabean farmers on a number of inexpensive agricultural or forest wastes such as *Dactyladenia barteri* and poultry manure individually and in combination.

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