Research Article

Management of the Disease Complex of Okra (*Abelmoschus esculentus* (L.) Moench) in Screenhouse caused by *Meloidogyne incognita* and *Sclerotium rolfsii* using *Trichoderma* Species and Neem Cake

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Abstract | Root-knot nematodes and fungi are serious pathogens affecting okra production. It has been observed that okra production has not been maximized due to nematode and fungus infestation. A research into the control of *Meloidogyne incognita* and *Sclerotium rolfsii* were examined in screenhouse. One gram of *Trichoderma viride* at 2.40×10^7 CFU/g and *Trichoderma harzianum* at 1.40×10^7 CFU/g of millet were applied in combination with neem cake at 2.00 t/ha and 4.00 t/ha. Soil not amended with neem cake and not infested with *Trichoderma* species served as control. Two weeks old okra plants were inoculated with 5,000 larvae of *M. incognita* and one gram of *S. rolfsii* (3 Sclerotia g^{-3}) in a heat sterilized soil. The obtained results showed highly susceptibility of okra to *M. incognita* and *S. rolfsii* infection without neem cake and *Trichoderma* species infestation. Amendment of soil with neem cake at 4.00t/ha in combination with *T. viride* significantly ($P\leq0.05$) reduced root galling, nematode population and nematode reproduction. Soil amendment with *Trichoderma* species significantly ($P\leq0.05$) reduced root galling, nematode population and nematode reproduction factor with *T. viride* having a higher efficacy than *T. harzianum*. *S. rolfsii* root colonization index showed that the colonization index was significantly ($P\leq0.05$) reduced in plants grown in soil amended with neem cake and *Trichoderma* species compared with the control. Growth and yield attributes were significantly ($P\leq0.05$) enhanced in plants amended with neem cake and *Trichoderma* species. The highest fresh pod yield was obtained on *T. viride* in combination with 4.00 t/ha of neem cake.

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Introduction

Okra (*Abelmoschus esculentus* (*L.*) moench), is an economically important vegetable crop grown in tropical and subtropical parts of the world. This crop is suitable for cultivation as a garden crop as well as on large commercial farms. India is the largest producer of okra in the world. It is also used as a vege-

table in Brazil, West Africa, and many other countries. In India, the major okra growing states are Uttar Pradesh, Bihar, and West Bengal (Eagri, 2023). The characteristics of okra adapts it to various uses, such as its fresh leaves, buds, flowers, succulent pods, soft stems and seeds (Mihretu *et al*., 2014). Its economic importance cannot be overemphasized, as it contains food nutrients such as proteins, calcium, vitamins C, and carbohydrates in larger quantities (Owolarafe and Shotonde, 2004). Okra seeds are also a good source of protein and oil and a substitute for coffee (Calisir and Yildiz, 2005).

The root-knot Nematodes are the major nematodes attacking okra worldwide, including Nigeria. They are widely spread, attacking virtually all crops and thereby limiting agricultural productivity (Agrios, 2005). *Meloidogyne incognita* is the major species affecting okra production. *Meloidogyne species* cause wilting, chlorosis, stunted growth, and the formation of galls in the roots of most crops, which often leads to destruction of roots, poor growth, poor yield, and crop failure when the nematode population exceeds economic threshold level (Fourie, 2018).

Southern blight (*Sclerotium rolfsii*) is an important soil- borne phytopathogenic fungus found in tropical and subtropical regions of the world (Buensanteai *et al*., 2012). The host range of *S. rolfsii* include potatoes, soybeans, sunflowers, tomatoes, okra, and cotton. The pathogen causes serious yield loss in these host organisms (Buensanteai *et al*., 2012). Synthetic fungicide remains the popular application to control *S. rolfsii*. Control is noted to be difficult to achieve without the use of synthetic fungicides. However, high level application of synthetic fungicides in agriculture has the potential to cause toxic effects on humans and wildlife as well as to cause environmental pollution (Heydari *et al*., 2015).

Different management strategies for plant-parasitic nematodes and *Sclerotium rolfsii* have been developed, some of which involve the use of botanicals, crop rotation, solarization, fungi such as *Trichoderma species,* and other biological control measures such as neem cake application (Whitehead, 1998). In a recent research, the combination of *Trichoderma species* with poultry manure was found to have greater potentials in reducing root galling and suppressing nematode population (Etim *et al*., 2023). This present study aims to manage disease complex of okra (*Abelmoschus esculentus* (L.) moench) in a screenhouse caused by *Meloidogyne incognita* and *Sclerotium rolfsii* using *Trichoderma* species and neem cake.

Materials and Methods

Experimental site and source of materials

This experiment was conducted in the screen house of the Department of Botany, University of Calabar, Nigeria. Okra seed (*Clemson spineless*) VGTH-014K (Premier brand seeds) were obtained from the National Institute for Horticultural Research (NIHORT) Ibadan, Oyo State, Nigeria. Stock culture of *Trichoderma* species were obtained from the Botany Department, University of Calabar. The neem cake used in the research was obtained from the Institute of Agricultural Research (IAR), Zaria, Kaduna State, Nigeria.

Soil analysis for physicochemical properties and pre-nematode density

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

Building up of the nematode population and inoculum preparation

A pure stock culture of *M. incognita* was multiplied on susceptible host (Cowpea – *Vigna unquiculata*) plants in the screen house planted in a steam-sterilized sandy-loam soil. The nematode population was built up at full maturity of the cowpea 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 the galled root was 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. Heavily galled roots of cowpea plants were uprooted at 8 weeks after planting and rinsed in tap water. The galled roots were cut into 1-2 cm segments for larvae extraction. Subsequently, the galled roots were placed in a blender and distilled water added, blended at a 5-seconds interval. The blended substrate was emptied into a 1000- ml beaker and more water was added and stirred. 30 ml of the suspension was poured into a nematode counting dish and the number of larvae was counted with the use of a stereomicroscope. An average of five counts gave approximately 5,000 sec-

ond stage juveniles per 30 ml of solution, which was the inoculum density required.

Collection and culturing of *Sclerotium rolfsii:* *S. rolfsii* was cultured water melon fruit. Infected fruits of water melon were collected from the Research Farm of Crop Science Department, Faculty of Agriculture, Forestry and Wildlife Resource Management University of Calabar, Nigeria, using polyethylene bags, laboratory gloves, masking tape, nose mask and a hand trowel.

Sterilization of Materials: The infected fruits were taken to the laboratory in sterilized polyethylene bags. The laboratory workbench was sterilized using sodium hypochlorite (bleach) solution and 99% ethanol. Cut sections of the diseased assay plant were surface sterilized with a 70% sodium hypochlorite solution for one minute and was rinsed immediately in distilled water, blotted dry on Whatman's No. 1 filter paper and placed on petri dishes. The wet steam method in an autoclave at 160^{0}C for 30 minutes was employed as the sterilization method in this research.

Preparation of media for culturing of Sclerotium rolfsii

Potato Dextrose Agar (PDA) was used to culture the fungus. The measured quantity of PDA (39g) was poured into 1000ml conical flask. 1000ml 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 wrapped again with aluminum foil up to the neck of the flask. The medium was placed in an autoclave at 121°C for 15 minutes. 500 milligrams of chloramphenicol was added to the solution, and allowed to cool before removing from the autoclave. Medium was poured into sterile petri dishes and labelled.

Isolation and identification of Sclerotium rolfsii

The sterilized cut sections of the diseased water melon fruit with brown Sclerotias on the entire surface were picked using a spatula and dropped in petri dishes containing potato dextrose agar (PDA) solution and labelled accordingly. The inoculated plates were incubated at a room temperature of 27± 1°C for 14days and daily observations were made for the emergence of fungal colonies.

To identify *Sclerotium rolfsii,* a drop of lacto phenol in cotton blue was used to stain the slide. A 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 slip for observation and identification under a light microscope (Olympus Optical Philippines) with x40 magnification. The morphological structures of the fungi were compared with those in the Atlas of Imperfect Fungi by Barnett and Hunter (1998) for identification. The colonies formed were subculture to obtain pure cultures of the isolate as shown in Plate 1 and 2.

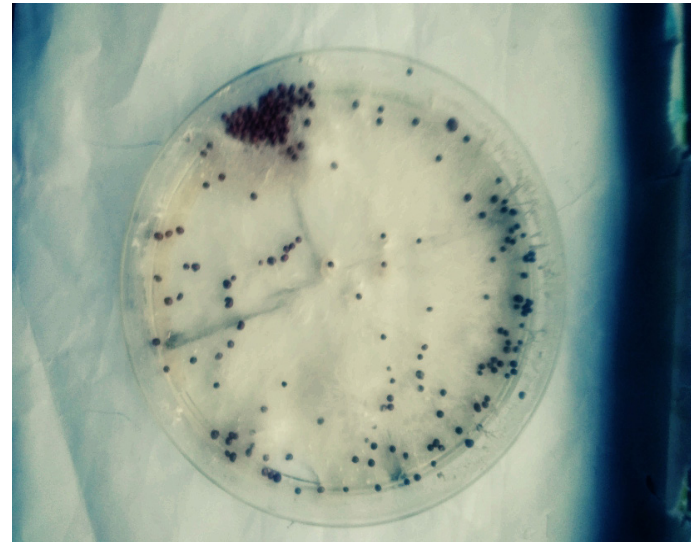

Plate 1: *Pure culture of Sclerotium rolfsii.*

Plate 2: *Photomicrograph of Sclerotium rolfsii.* MAG. X 400

Preparation of carrier for Sclerotium rolfsii

The preparation of carriers for *Sclerotium rolfsii* was carried out according to Sivan *et al.* (1984). 5g of millet grain was weighed using a sensitive weighing balance. The millet was soaked in water for 24 hours to ferment before used. Normal sterilization of mil-

let grains inside the bottles was done using an autoclave at the range of 121°C for 15 minutes. The millet grains in the sterilized Bima bottles were allowed to cool before using a spatula to pick up three sclerotia and add them it to the subsrates. 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 allowed to grow and sporulate in bottles up to 14 days for Sclerotia to form.

Sub culturing and spore count of Trichoderma species

Spore count of *Trichoderma* species was carried out according to Pitt and Poole (1981). The pure stock culture of the two species of *Trichoderma* (*T. viride* and *T. harzianum*) were sub cultured on Potato Dextrose Agar (PDA). To quantify colony form unit (CFU/g), both species of *Trichoderma in* Petri dishes were scraped and 1 gram of each species was weighed using an Ohaus sensitive weighing balance. The weighed substrates were poured into a conical flask, and 10 ml of distilled water was added to the substrates, stirred using a magnetic stirrer and, later transferred to a vortex mixer to obtain homogenous mixture. 0.05ml of the diluted samples were transferred with the help of a pipette into the cell of the hemacytometer for counting using binocular microscope. The spore concentration was determined as millions of spores CFU per grams of millet sample, and the number of spores generated serves as the inoculum load.

The spores counted were as follows;

1. *Trichoderma viride* gave 2.40 x10^7 CFU/g of millet which was used for inoculation in screenhouse.
2. *Trichoderma harzianum* gave 1.40 x10^7 CFU/g of millet which was used for inoculation in screenhouse experiment.

Preparation of carrier for Trichoderma species

The preparation of carrier for *Trichoderma species* was carried out according to Sivan *et al*. (1984). 200g 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 by autoclaving at 121° c for 15 minutes. The millet grains in the sterilized Bema bottles were allowed to cool and a sterilized cork borer (five mycelia discs) of different species of *Trichoderma* grown on PDA as pure culture was added to the 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. Substrates were incubated at room temperature in the laboratory for 3 - 5 days. After that, the substrates were taken immediately to screenhouse for inoculation.

Experimental layout, data collection and statistical analysis

The experiment was laid out as a 4×3×3 factorial experiment fitted into a Completely Randomized Design (CRD) with having 36 treatment combinations and three replicates to give a total of 108 experimental units. Data were collected at a two weeks interval starting from three weeks after planting (3WAP) on the following: Number of galls per plant, gall index, number of nematode per plant, nematode density in soil, nematode reproduction factor, *Sclerotium rolfsii* root colonization, plant height, number of leaves, number of pods, fresh pod weight per plant, 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 compared using the Duncan New Multiple Range Test (DNMRT) at a 5% probability level. However, for nematode infection incidence and *Sclerotium rolfsii* root colonization index results, the main effects of pathogens were compared using F – LSD (0.05) at 5% probability.

Application of Neem cake and planting of okra seeds

Neem cake was applied at a rate of 10g and 20g per planting pot, equivalent to 2 and 4t/ha on designated pots respectively except for the uninoculated pots, which serves as controls. The neem cake was applied two weeks before planting by thoroughly mixing with soil in the pots. Each pots was sown with three seeds of okra (Clemson spineless) at a depth of 3 cm per pot 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 screenhouse in a completely randomized designed. The normal spacing of 30cm between rows and 30cm within rows was maintained. At 2WAP, the soil around the plant was excavated to a depth of 5cm and a nematode suspension of 30ml (5,000 infective juvenile) was inoculated into the soil. The soil around the roots was covered immediately.

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Inoculation of a potted okra plant with Sclerotium rolfsii
The prepared millet grain carrier of 200 grammes in Bema bottles was taken to the screenhouse for inoculation into pots. 3cm holes were made around the base of okra plants and one gramme (3 Sclerotias g^{-3}) was used to inoculate pots receiving treatment of *Sclerotium rolfsii* alone, or *Meloidogyne incognita* in combination with *Sclerotium rolfsii*. The uninoculated ones served as control. The inoculated areas were covered with soil immediately.

Inoculation of potted okra plant with Trichoderma species
The prepared millet grains carrier of 200 grams in Bema bottles with the inoculated spores of two *Trichoderma species* was taken to screenhouse for inoculation into pots. 3cm holes were made around the base of the okra plant and one gramme (1g) of each species of *Trichoderma* was used to inoculate in all pots receiving treatments except the un-inoculated ones which served as control. The inoculated areas were covered with soil immediately after inoculation.

Results and Discussion

Main effects of neem cake, Trichoderma species and their interactions on the number of galls per plant and gall index (GI) per okra plants inoculated with Meloidogyne incognita and Sclerotium rolfsii.
Table 1 shows that okra plants inoculated with both pathogens (*M. incognita* and *S. rolfsii*) significantly (P≤0.05) had more root galls than inoculation with only *M. incognita*. A successive increase in the rate of neem cake significantly (P≤0.05) led to decrease in root galling in okra. Soil amended with 4.00t/ha neem had plants with the least number of galls. Application of *Trichoderma* species significantly (P≤0.05) reduced number of galls compared with the control. However, *T. viride* treated plants had the least number of galls. The gall index followed the trend of the number of galls. Concomitant inoculation of both pathogens produced plants with a higher gall index than inoculation with only *M. incognita*. An increase in the neem cake rate significantly reduced the gall index. Both species of *Trichoderma* significantly reduced gall index compared with no application with *T. viride* inoculated plants having the least gall index. The interaction effects of treatments on the number of galls per plant and gall index shows that combined inoculation of the okra plant with *M. incognita and S. rolfsii* without neem cake amendment had plants with significantly (P≤0.05) the highest number of galls, followed by *M. incognita,* without neem treatment. Neem cake amendment significantly (P≤0.05) reduced root galling, with 4.00 t/ha neem cake having the greatest reduction effect.

Table 1: *Main effects of neem cake (N.) and Trichoderma species (T.) on the number of galls per plant and gall index (GI) per okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.*

Neem (t/ha	Number of galls per plant	Gall index
N0 0.00	115.89±10.71[a]	4.50±0.12[a]
N1 2.00	38.33±3.69[b]	3.67±0.11[b]
N2 4.00	14.44±1.11[c]	2.78±0.10[c]
***Trichoderma* species (T.)**		
T0 (control)	81.89±16.43[a]	4.00±0.20[a]
TI *Trichoderma viride*	38.67±7.39[c]	3.28±0.20[c]
T2 *Trichoderma Harzianum*	48.11±8.68[b]	3.67±0.18[b]
Pathogens (P.)		
P1. *Meloidogyne incognita* alone	47.44+8.99	3.44+0.16
P3. *Meloidogyne incognita + Sclerotium rolfsii*	65.00+10.67	3.85+0.16
F-LSD (0.05)	2.75	0.08

** Means within a column followed by the same letter of the alphabets are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at the 5% probability level with respect to the main effect of factors.*

Table 2 shows that the interaction between *Trichoderma* and neem cake was significant. Plants not inoculated with *Trichoderma* and without the neem cake amendment had significantly (P≤0.05) highest number of galls. However, neem cake amendment in combination with *Trichoderma* species inoculation significantly inhibited root galling in okra, with 4.00t/ha neem in combination with either species of *Trichoderma* having the least number of galls. The interaction effects of pathogens, neem and *Trichoderma* was significant with regards to gall index (GI). Inoculation of *M. incognita* alone or *M. incognita* in combination with *S. rolfsii* without *Trichoderma* and neem cake amendment had plants with significantly highest gall index. Inoculation of okra plants with *T. viride* in combination with neem cake amendment at 4.00t/ha produced plants with significantly (P≤0.05) least gall index.

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Table 2: *Interaction effects of neem cake (N.) and Trichoderma species (T.) on the number of galls per plant and gall index per plant of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.*

Treatment combinations	Number of galls per plant	Treatment combinations	Gall index
(T×N)			
T0N0	173.50±8.51[a]	P1T0N0	5.00± 0.00[a]
T1N0	78.67±5.57[c]	P1TIN0	4.00± 0.00[b]
T2N0	95.50±6.53[b]	P1T2N0	4.00±0.00[b]
T0N1	54.00±5.42[d]	P3T0N0	5.00±0.00[a]
T1N1	26.83±4.28[f]	P3T1N0	4.00±0.00[b]
T2N1	34.17±3.50[e]	P3T2N0	5.00±0.00[a]
T0N2	18.17±2.15[g]	P1T0N1	4.00±0.00[b]
T1N2	10.50±0.89[h]	P1T1N1	3.00±0.00[c]
T2N2	14.67±1.15[gh]	P1T2N1	3.00±0.00[c]
(P×N)		P3T0N1	4.00+0.00[b]
P1N0	101.78±14.23[b]	P3T1N1	4.00±0.00[b]
P3N0	130.00±15.34[a]	P3T2N1	4.00±0.00[b]
P1N1	28.89±3.66[d]	P1T0N2	3.00±0.00[c]
P3N1	47.78±4.71[c]	P1T1N2	2.00+0.00[e]
P1N2	11.67±0.80[f]	P1T2N2	3.00+0.00[c]
P3N2	17.22±1.64[e]	P3T0N2	3.00+0.00[c]
		P3T1N2	2.67+0.33[c]
		P3T2N2	3.00+0.00[c]

**Means within a column followed by the same letter of the alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at the 5% probability level.*
P0: *No pathogens (control);* **P1:** *Meloidogyne incognita alone;* **P2:** *Sclerotium rolfsii alone;* **P3:** *Meloidogyne incognita + Sclerotium rolfsii.*
N0: *No neem cake (control);* **N1:** *Neem cake at 2 t/ha;* **N2:** *Neem cake at 4 t/ha.*
T0: *No Trichoderma (control);* **T1:** *Trichoderma viride;* **T2:** *Trichoderma harzianum.*

Main effect of neem cake, Trichoderma species and their interactions on the number of nematodes per plant, nematode density in soil and nematode reproduction factor (R_f) of okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.
Table 3 shows that okra plants inoculated with both pathogens (*M. incognita* and *S. rolfsii*) significantly (P≤0.05) had a higher number of nematodes per plant than when inoculated with only *M. incognita* alone. The increase in the rate of neem cake significantly (P≤0.05) reduced the number of nematodes per plant compared with the control. Soil amended with 4.00t/ha of neem cake had plants with the least number of nematodes per okra plant. Application of *Trichoderma* species significantly (P≤0.05) reduced number of nematodes per plant compared with the control. However, plants treated with *T. viride* had the least number of nematodes per plant.

Nematode density in soil and nematode reproduction factor (R_F) followed the trend of number of nematodes per plant. Concomitant inoculation of both pathogens (*M. incognita* and *S. rolfsii)* significantly (P≤0.05) had the highest nematode density in soil and nematode reproduction factor than when inoculated with *M. incognita* alone. A successive increase in neem cake significantly (P≤0.05) reduced nematode density in soil and nematode reproduction factor (R_f). Soil amended with 4.00t/ha of neem had the least nematode density and nematode reproduction factor in soil. However, both Species of *Trichoderma* significantly reduced nematode density in soil as well as nematode reproduction factor compared with no application with *T. viride* inoculated plants having the least nematode density in soil and nematode reproduction factor.

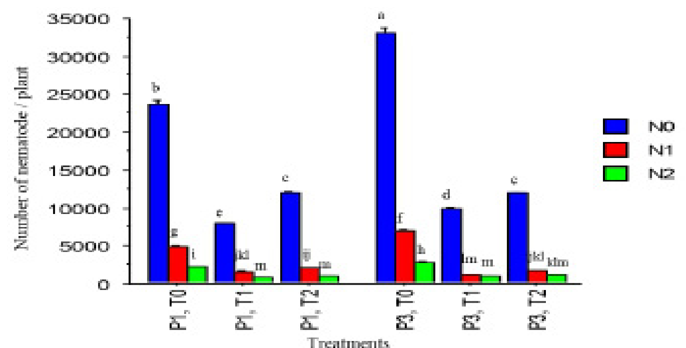

Figure 1: *Interaction effects of neem cake (N.) and Trichoderma species (T.) on the number of nematodes per plant.*
P0: *No pathogens (control);* **P1:** *Meloidogyne incognita alone;* **P2:** *Sclerotium rolfsii alone;* **P3:** *Meloidogyne incognita + Sclerotium rolfsii.*
N0: *No neem cake (control);* **N1:** *Neem cake at 2 t/ha;* **N2:** *Neem cake at 4 t/ha.*
T0: *No Trichoderma (control);* **T1:** *Trichoderma viride;* **T2:** *Trichoderma harzianum.*

Table 4 and Figure 1, shows that the combined inoculation of okra plants with *M. incognita* and *S. rolfsii* without *Trichoderma* species and neem cake amendment (P3T0N0) significantly (P<0.05) had the highest number of nematodes per plant, followed by plants inoculated with only *M. incognita* without *Trichoderma* species and neem cake (P1T0N0). Inter

Table 3: *Main effects of neem cake (N.) and Trichoderma species (T.) on the number of nematodes per plant, nematode density in soil, and nematode reproduction factor (R_f) of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.*

Neem (t/ha)	Number of nematode per plant	Nematode density in soil	Nematode reproduction factor
(N0) 0.00	16448.00±2185.60[a]	190994.00±30647.35[a]	41.49±6.55[a]
(N1) 2.00	3083.00± 519.40[b]	39815.00± 5130.76[b]	8.58±1.13[b]
(N2) 4.00	1499.00±184.16[c]	1185.00±982.76[c]	2.67±0.23[c]
***Trichoderma* Species (T.)**			
T0 (control)	12307.00±2868.10[a]	151647.00±37701.65[a]	32.79±8.11[a]
T1 *Trichoderma viride*	3734.00±903.83[c]	42085.00±9382.67[c]	9.16±2.06[c]
T2 *Trichoderma harzianum*	4988.00±1203.57[b]	48929.00± 10327.69[b]	10.78±2.30[b]
Pathogens (P.)			
(P1.) *Meloidogyne incognita*	6258.00± 1395.87	76434.00±20478.70	16.54±4.37
(P3.) *Meloidogyne incognita +Sclerotium rolfsii*	7761.00± 1918.55	85340.00± 21834.23	18.62±4.75
F-LSD (0.05)	181.00	966.40	0.21

** Means within a column followed by the same letter of the alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at the 5% probability level with respect to the main effect of factors.*

Table 4: *Interaction effects of neem cake (N.) and Trichoderma species (T.) on the nematode reproduction factor (R_f) of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii*

Treatment Combinations	Nematode reproduction factor
P_1 T0N0	75.81±0.72[b]
P_1 T1N0	19.37± 0.22[f]
P_1 T2N0	23.41± 0.15[d]
P_3 T0N0	83.18±0.29[a]
P_3 T1N0	22.52± 0.33[e]
P_3 T2N0	24.62± 0.12[c]
P_1 T0N1	13.91± 0.19[h]
P_1 T1N1	4.23± 0.05[k]
P_1 T2N1	5.21± 0.03[j]
P_3 T0N1	16.08±0.10[g]
P_3 T1N1	5.22± 0.04[j]
P_3 T2N1	6.84±0.07[i]
P_1 T0N2	3.40±0.03[l]
P_1 T1N2	1.52± 0.03[n]
P_1 T2N2	1.98±0.04[mn]
P_3 T0N2	4.35±0.04[k]
P_3 T1N2	2.13± 0.04[mn]
P_3 T2N2	2.64±0.03[m]

** Means within a column followed by the same letter of the alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at the 5% probability level.*
For key please see Table 1.

action effects between *Trichoderma* species and neem cake was significant (P≤0.05) in reducing the number of nematodes per okra plant. However, plants inoculated with *M. incognita,* alone treated with *T. viride,* and amended with 4.00t/ha of neem cake (P1T1N2) had significantly (P≤0.05) the least number of nematodes per plant.

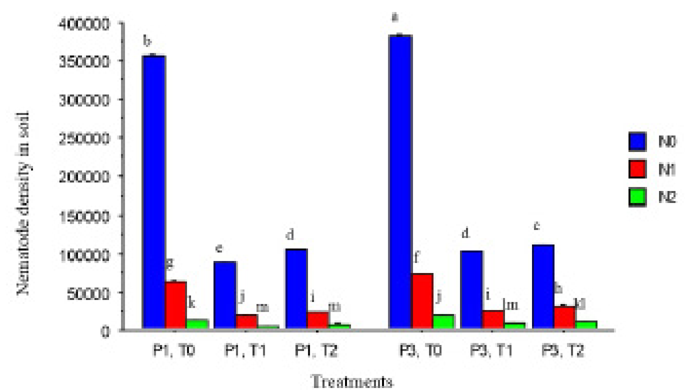

Figure 2: *Interaction effects of neem cake (N.) and Trichoderma species (T.) on the nematodes density in soil. For key please see Figure 1.*

Figure 2 shows that there were also significant interactions between pathogens, *Trichoderma* species, and neem cake on nematode density in soil. Soils inoculated with *M. incognita* and *S. rolfsii* without *Trichoderma* species and neem cake (P3T0N0) had significantly (P≤0.05) the highest nematode density in soil, followed by plants inoculated with only *M. incognita* without *Trichoderma* and neem cake (P1T0N0). The

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interaction effects between *Trichoderma* species and neem cake were also significant (P≤0.05) in reducing nematode density in soil. However, okra plants inoculated with *M. incognita* alone, treated with *T. viride*, and amended with 4.00t/ha of neem cake (P1T1N2) had significantly (P≤0.05) the least soil nematode density.

Nematode reproduction factor (R_f) followed the trend of nematode density in soil. Concomitant inoculation of both pathogens *M. incognita* and *S. rolfsii* without *Trichoderma* species and neem cake amendment (P3T0N0) significantly (P<0.05) had the highest nematode reproduction factor followed by plants inoculated with only *M. incognita* alone without *Trichoderma* species and neem cake (P1T0N0). However, plants inoculated with *M. incognita* treated with *T. viride* and amended with 4.00t/ha of neem cake (P1T1N2) had significantly (P≤0.05) the least nematode reproduction factor.

Main effects of neem cake, Tricoderma species and their interactions on Sclerotium rolfsii root colonization index of okra inoculated with Meloidogyne incognita and Sclerotium rolfsii.

Table 5 shows that *Sclerotium rolfsii* root colonization index was significantly (P≤0.05) lower in okra plants inoculated with *S. rolfsii* alone, (P2) than when *M. incognita* and *S. rolfsii* were combined (P3). A successive increase in the rate of neem cake significantly (P≤0.05) reduced *Sclerotium rolfsii* root colonization index in the okra plant. Soil amended with 4.00t/ha of neem cake significantly (P≤0.05) reduced *Sclerotium rolfsii* root colonization index compared with the control. Following the application of *Trichoderma* species, the *Sclerotium rolfsii* root colonization index was reduced with *T. viride* treated plants having the lowest *Sclerotium rolfsii* root colonization index. The interaction effects of treatment on *Sclerotium rolfsii* root colonization index. There were significantly (P≤0.05) higher *Sclerotium rolfsii* root colonization indexes on okra plants inoculated with *Sclerotium rolfsii* alone, or *M. incognita* in combination with *S. rolfsii* without *Trichoderma* species and neem cake (P2T0N0 and P3T0N0). However, amendment of soil with *Trichoderma* species and neem cake had significant interaction effects on the *Sclerotium rolfsii* root colonization index.

Main effects of neem cake, Trichoderma species and their interactions on plant height (cm) at 3 weeks after planting (WAP) of an okra plant inoculated with Meloidogyne incognita and
Sclerotium rolfsii

Table 6 shows that okra plants inoculated with *M. incognita* alone (P1), *S. rolfsii* alone (P2), or *M. incognita* in combination with *S. rolfsii* (P3) did not have significant (P≥0.05) effects on plant height at 3WAP. The increase in the rate of neem cake significantly (P≤0.05) enhanced plant height than the control. Okra plants amended with 4.00t/ha of neem cake significantly (P≤0.05) had higher plant heights than 2.00t/ha of soil amendment. Application of *Trichoderma* species (T1 and T2) did not have a significant (P≥0.05) increase in plant height compared with the control.

The interaction effects of treatments between *Trichoderma* species and neem cake was significant (P≤0.05) on plant height at 3 weeks after planting with the tallest plants obtained when T2N2 were combined. Interaction between pathogens and neem cake had significant effects on plant height, with the tallest plants obtained when P3N2 were combined. However, interaction between pathogens and *Trichoderma* species also had significant effects on plant height at 3 weeks after planting. Although, there was more uniformity in heights compared to T×N and P×N interactions.

Main effects of neem cake, Trichoderma species, and their interactions on Plant height (cm) of okra plants inoculated with Meloidogyne incognita and Sclerotium rolfsii at 5 and 7 weeks after planting

Table 7 shows that inoculation of okra plants with pathogens significantly (P≤0.05) reduced plant heights at 5 and 7 weeks after planting compared with the control. Okra plants inoculated with both pathogens *M. incognita* and *S. rolfsii* (P3) significantly (P≤0.05), had the shortest heights at 5 and 7 weeks after planting. Amendment of soil with neem cake and *Trichoderma* species significantly enhanced plant heights. Soil amended with neem cake at 4.00 t/ha at both 5 and 7 weeks after planting significantly produced plants with the tallest height. Treatment of okra plants with *Trichoderma* species enhanced plant heights at 5 and 7 (WAP). However, application of *T. viride* at 5 and 7 weeks after planting significantly produced the tallest okra plant.

Table 8 shows that the combined inoculation of okra plants with *M. incognita* alone without *Trichoderma* species and neem cake (P1T0N0) or *M. incognita* in

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Table 5: *Main effects neem cake (N.), Trichoderma species (T.) and their interactions on the Sclerotium rolfsii root colonization index of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.*

1		Treatment Combinations	Sclerotium rolfsii root colonization index
N0 0.00	1.67±0.18[a]	P2T0N0	2.00±0.00[b]
N1 2.00	1.39±0.12[b]	P2T1N0	1.00±0.00[d]
N2 4.00	1.28±0.11[b]	P2T2N0	1.00±0.00[d]
		P3T0N0	3.00±0.00[a]
***Trichoderma* Species (T.)**		P3T1N0	1.00± 0.00[d]
T0 (control)	2.11±0.11[a]	P3T2N0	2.00± 0.00[b]
T1 *Trichoderma viride*	1.00±0.00[c]	P2T0N1	2.00±0.00[b]
T2 *Trichoderma harzianum*	1.22±0.10[b]	P2T1N1	1.00±0.00[d]
		P2T2N1	1.00±0.00[d]
		P3T0N1	2.00±0.00[b]
		P3T1N1	1.00±0.00[d]
Pathogens (P.)	1.30+0.09	P3T2N1	1.33±0.33[d]
	1.59+0.13	P2T0N2	1.67±0.33[c]
(P2.) *Sclerotium rolfsii alone*	0.11	P2T1N2	1.00±0.00[d]
(P3.) *M. incognita +S. rolfsii*		P2T2N2	1.00±0.00[d]
F-LSD (0.05)		P3T0N2	2.00±0.00[b]
		P3T1N2	1.00±0.00[d]
		P3T2N2	1.00±0.00[d]

** Means within a column followed by the same letter of the alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at the 5% probability level with respect to the main effects of factors.*
For key please see Table 1.

Table 6: *Main effects of neem cake (N.), Trichoderma species and their interactions on the plant height (cm) at 3 weeks planting (WAP) of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.*

Neem (t/ha)		TxN (interactions)	PxN (interactions)	PxT (interactions)
N0 0.00	13.54±0.13[c]	T0N0 13.72±0.16[d]	P0N0 13.97±0.17[e]	P0T0 16.24±0.71[b]
N1 2.00	16.88+0.10[b]	T1N0 13.99±0.17[d]	P1N0 13.61±0.32[ef]	P0T1 16.71±0.71[ab]
N2 4.00	19.38+0.09[a]	T2N0 12.92±0.20[e]	P2N0 13.24±0.26[f]	P0T2 16.58±0.88[ab]
		T0N1 16.93±0.23[c]	P3N0 13.36±0.20[f]	P1T0 16.40±0.74[ab]
***Trichoderma* species (T.)**		T1N1 16.96±0.07[c]	P0N1 16.40±0.23[d]	P1TI 16.89±0.70[a]
				P1T2 16.56±1.04[ab]
To Control	1.60+0.39[a]	T2N1 16.77±0.21[c]	P1N1 17.06±0.13[c]	P2T0 16.92±0.88[a]
		T0N2 19.16±0.16[b]	P2N1 17.08±0.17[c]	P2T1 16.47±0.82[ab]
T1 *Trichoderma viride*	16.73±0.37[a]	TIN2 19.23±0.13[b]	P3N1 17.00+0.24[c]	P2T2 16.43+1.09[ab]
T2 *Trichoderma harzianum*	16.48±0.48[a]	T2N2 19.76±0.14[a]		P3T0 16.83+0.91[a]
			P0N2 19.14+0.16[b]	P3T1 16.84+0.85[a]
			P1N2 19.18+0.20[b]	P3T2 16.39+1.03[ab]
			P2N2 19.50+0.21[ab]	
Pathogens (P.)			P3N2 19.71+0.95[a]	
Po. (control)	16.50±0.43[a]			
P1. *Meloidogyne incognita* alone	16.61±0.47[a]			
P2. *Sclerotium rolfsii* alone	16.61±0.52[a]			
P3. *M. incognita* + *S. rolfsii*	16.69±0.52[a]			

**Means within a column followed by the same letter of the alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at the 5% probability level.*
For key please see Table 1.

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Table 7: *Main effects of neem cake (N.) and Trichoderma species (T.) on the plant height (cm) at 5 and 7 weeks after planting (WAP) of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.*

Neem (t/ha)	5 WAP	7 WAP
N0 0.00	17.11±0.27^{c}	16.37±1.25^{c}
N1 2.00	21.79±0.30^{b}	23.69±0.31^{b}
N2 4.00	25.32±0.43^{a}	27.25±0.43^{a}
***Trichoderma* species (T.)**		
To (Control)	20.18±0.66^{c}	19.47±1.55^{c}
T1. *Trichoderma viride*	22.64±0.66^{a}	24.64±0.67^{a}
T2. *Trichoderma harzianum*	21.40±0.61^{b}	23.20±0.60^{b}
Pathogens (P.)		
Po (control)	23.32±0.94^{a}	25.55±0.81^{a}
P1 *Meloidogyne incognita* alone	20.76±0.66^{c}	22.72±0.65^{b}
P2 *Sclerotium rolfsii* alone	21.66±0.76^{b}	21.46±1.64^{c}
P3 *M. incognita* +*S. rolfsii*	19.89±0.48^{d}	20.01±1.43^{d}

**Means within a column followed by the same letter of the alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at the 5% probability level.*

Table 8: *Interaction effects of neem cake (N.) and Trichoderma species (T.) on the plant height (cm) at 5 and 7 weeks after planting (WAP) of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.*

Treatment Combination	5 WAP	7 WAP
P0T0N0	14.07±0.07^{q}	20.77±0.41^{n}
P0T1N0	19.60±0.49^{jk}	20.55±0.51^{n}
P0T2N0	18.90±0.25^{kl}	20.21±0.21^{no}
P1T0N0	14.97±0.19^{p}	17.09±0.49^{r}
P1T1N0	17.67±0.27^{n}	19.86±0.26^{nop}
P1T2N0	16.60±0.21^{o}	18.47±0.13^{q}
P2T0N0	17.17±0.18^{no}	0.00±0.00^{s}
P2T1N0	17.83±0.17^{mn}	19.95±0.05^{nop}
P2T2N0	17.13±0.18^{no}	19.28±0.25^{p}
P3T0N0	15.33±0.57^{p}	0.00±0.00^{s}
P3T1N0	18.53±0.26^{lm}	20.67±0.17^{n}
P3T2N0	17.53±0.13^{n}	19.54±0.14^{op}
P0T0N1	23.17±0.20^{ef}	24.88±0.05^{fgh}
P0T1N1	25.60±0.25^{cd}	27.62±0.31^{cd}
P0T2N1	24.03±0.12^{e}	25.87±0.33^{e}
P1T0N1	21.13±0.41^{ghi}	22.97±0.29^{kl}
P1T1N1	23.00±0.29^{f}	25.01±0.29^{fg}
P1T2N1	21.47±0.15^{gh}	23.55±0.14^{jk}
P2T0N1	20.03±0.18^{j}	22.09±0.17^{m}
P2T1N1	22.00±0.29^{g}	24.07±0.29^{hij}
P2T2N1	20.93±0.29^{hi}	21.96±0.28^{m}
P3T0N1	19.73±0.34^{jk}	21.75±0.33^{m}
P3T1N1	20.43±0.18^{ij}	22.64±0.13^{lm}
P3T2N1	19.90±0.51^{j}	21.85±0.25^{m}
P0T0N2	25.83±0.30^{cd}	27.85±0.30^{cd}
P0T1N2	29.97±0.19^{a}	32.14±0.28^{a}
P0T2N2	28.70±0.21^{b}	30.07±0.48^{b}
P1T0N2	23.23±0.22^{ef}	24.62±0.23^{ghi}
P1T1N2	25.13±0.29^{d}	27.17±0.30^{d}
P1T2N2	23.63±0.29^{ef}	25.72±0.35^{ef}
P2T0N2	25.57±0.32^{cd}	27.60±0.35^{cd}
P2T1N2	28.00±0.31^{b}	30.02±0.32^{b}
P2T2N2	26.27±0.32^{c}	28.17±0.39^{c}
P3T0N2	21.93±0.34^{g}	23.99±0.31^{ij}
P3T1N2	23.90±0.17^{e}	25.92±0.17^{e}
P3T2N2	21.73±0.34^{gh}	23.74±0.33^{jk}

** Means within a column followed by the same letter of the alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at the 5% probability level.*
For key please see Table 1.

combination with *S. rolfsii* without *Trichoderma* species and neem cake (P3T0N0) had significantly the lowest height at 5 weeks after planting.

However, interaction effects between *Trichoderma* species and neem cake on plant height in all other pots at 5 and 7 weeks after planting significantly ($P \leq 0.05$) enhanced plant height with the tallest plants obtained when (P0T1N2) interacted. Comparatively, plant heights were increase as the number of weeks increased.

Main effects of neem cake, Trichoderma species and their interactions on the plant height (cm) of okra plants inoculated with Meloidogyne incognita and Sclerotium rolfsii at 5 and 7 weeks after planting

Table 7 shows that inoculation of okra plants with pathogens significantly ($P \leq 0.05$) reduced plant heights at 5 and 7 weeks after planting compared with the control. Okra plants inoculated with both pathogens, *M. incognita* and *S. rolfsii* (P3) significantly ($P \leq 0.05$), had the shortest heights at 5 and 7 weeks after planting. Amendment of soil with neem cake and *Trichoderma* species significantly enhanced plant heights. Soil amended with neem cake at 4.00 t/ha at both 5 and 7 weeks after planting significantly produced plants with the tallest height. Treatment of

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Table 9: *Main effects of neem cake (N.), Trichoderma species (T.) and their interactions on the number of leaves at 3 Weeks after planting (WAP) of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.*

Neem (t/ha)	**Treatment 3 WAP**				
N0 0.00	5.14±0.11^{c}	P0T0N0	4.33±0.33^{a}	P2T1N1	6.00±0.58^{a}
N1 2.00	6.50±0.11^{b}	P0T1N0	4.67±0.33^{a}	P2T2N1	6.67±0.33^{a}
N2 4.00	7.86±0.12^{a}	P0T2N0	5.00±0.58^{a}	P3T0N1	6.67±0.33^{a}
		P1T0N0	5.33±0.33^{a}	P3T1N1	5.67±0.33^{a}
***Trichoderma* species (T.)**		P1T1N0	5.33+0.33^{a}	P3T2N1	7.33±0.33^{a}
		P1T2N0	5.00+0.00^{a}		
T0 (control)	6.42±0.21^{a}	P2T0N0	5.67+0.33^{a}	P0T0N2	7.67±0.33^{a}
T1 *Trichoderma viride*	6.42±0.22^{a}	P2T1N0	5.00+0.00^{a}	P0T1N2	7.33±0.33^{a}
T2 *Trichoderma harzianum*	6.67±0.23^{a}	P2T2N0	5.33±0.33^{a}	P0T2N2	8.33±0.33^{a}
		P3T0N0	5.00+0.58^{a}	P1T0N2	7.67±0.33^{a}
		P3T1N0	5.67+0.33^{a}	P1T1N2	8.00±0.58^{a}
		P3T2N0	5.33+0.33^{a}	P1T2N2	8.00±0.58^{a}
		P0T0N1	6.33+0.33^{a}	P2T0N2	8.00±0.58^{a}
Pathogens (P.)		P0T1N1	6.67+0.33^{a}	P2T1N2	8.33±0.33^{a}
P0 (control)	6.30+0.8^{a}	P0T2N1	6.33+0.33^{a}	P2T2N2	8.00±0.58^{a}
P1. *Meloidogyne incognita* alone	6.56+0.25^{a}	P1T0N1	6.33+0.33^{a}	P3T0N2	7.33±0.33^{a}
P2. *Sclerotium rolfsii* alone	6.63+0.26^{a}	P1T1N1	6.33+0.33^{a}	P3T1N2	8.00±0.58^{a}
P3. *M. incognita* + *S. rolfsii*	6.52+0.24^{a}	P1T2N1	7.00+0.00^{a}	P3T2N2	7.67±0.33^{a}
		P2T0N1	6.67+0.33^{a}		

** Means within a column followed by the same letter of the alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNRMT) at the 5% probability level.*
For key please see Table 1.

okra plants with *Trichoderma* species enhanced plant heights at 5 and 7 (WAP). However, application of *T. viride* at 5 and 7 weeks after planting significantly produced the tallest okra plant.

Table 8 shows that the combined inoculation of okra plants with *M. incognita* alone without *Trichoderma* species and neem cake (P1T0N0) or *M. incognita* in combination with *S. rolfsii* without *Trichoderma* species and neem cake (P3T0N0) had significantly the lowest height at 5 weeks after planting.

However, interaction effects between *Trichoderma* species and neem cake on plant height in all other pots at 5 and 7 weeks after planting significantly ($P \leq 0.05$) enhanced plant height with the tallest plants obtained when (P0T1N2) interacted. Comparatively, plant heights were increase as number of weeks increased.

Main effects of neem cake, Trichoderma species, and their interactions on number of leaves per plant at 3 weeks after planting (WAP) of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii

Table 9 shows that okra plants inoculated with the pathogens *M. incognita* alone (P1), *S. rolfsii* alone (P2), or *M. incognita* in combination with *S. rolfsii* (P3) did not have significantly ($P \geq 0.05$) effects on the number of leaves at 3 weeks after planting. However, application of neem cake significantly ($P \leq 0.05$) enhanced leaf production in okra plants, with the highest number of leaves obtained from plants treated with 4.00t/ha of neem cake. Application of *Trichoderma* species did not have a significant ($P \geq 0.05$) increase in number of leaves at 3WAP. Also, the interaction between the pathogens, *Trichoderma* species, and neem cake was not significant in terms of the number of leaves at 3 weeks after planting.

Main effects of neem cake, Trichoderma species, and their interactions on number of leaves at 5 and 7 weeks after planting (WAP) of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.

Table 10 shows that at 5 and 7 weeks after planting, okra plants inoculated with *M. incognita* alone (P1), *S. rolfsii* alone (P2) or *M. incognita* in combination with *S. rolfsii* (P3) significantly ($P \leq 0.05$) had lower leaf production compared with the control. Application of neem cake significantly ($P \leq 0.05$) enhanced leaf production at 5 and 7 weeks after planting with 4.00

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t/ha treated plants having the highest leaf production. Okra plants treated with *T. viride* significantly (P≤0.05) had higher number of leaves production at 5 and 7 weeks after planting than *T. harzianum.*

Table 10: *Main effects of neem cake (N.) and Trichoderma species (T.) on the number of leaves at 5 and 7 weeks after planting (WAP) of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.*

Neem (t/ha)	5 WAP	7 WAP
N0 0.00	6.58+0.19[c]	7.83+0.62[c]
N1 2.00	8.72+0.20[b]	10.92+0.23[b]
N2 4.00	9.67+0.28[a]	12.06+0.42[a]
Trichoderma species (T.)		
T0 (control)	7.14±0.28[c]	8.22±0.66[c]
T1 *Trichoderma viride*	9.22±0.27[a]	11.78±0.38[a]
T2 *Trichoderma harzianum*	8.61±0.28[b]	10.81±0.33[b]
Pathogens (P.)		
P0 (control)	9.41+0.36[a]	12.70+0.47[a]
P1 *Meloidogyne incognita* alone	7.74+0.25[b]	9.81+0.26[b]
P2 *Sclerotium rolfsii* alone	8.07+0.39[b]	9.33+0.72[c]
P3 *M. incognita + S rolfsii*	8.07+0.34[b]	9.22+0.69[c]

** Means within a column followed by the same letter of the alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at the 5% probability level.*

Table 11 shows that at 5 weeks after planting, interaction effects between pathogens and neem cake (P×N) was significant on number of leaves compared with the control (P0N0). The highest number of leaves produced per okra plant were obtained when P0N2 treatments were combined at 5 weeks after planting. Interaction effects between pathogens and *Trichoderma* species (P×T) on the number of leaves at 5WAP was also significant compared with the control (P0T0). However, the highest number of leave production per okra plants was obtained when P0T1 and 5WAP were combined at 5WAP.

At 7 weeks after planting, plants inoculated with *M. incognita* alone without *Trichoderma* species and neem cake amendment (P1T0N0) significantly (P≤0.05) had plants with the lowest number of leaf production compared with the control. However, inoculation of okra plants with *T. viride* in combination with neem cake at 4.00 t/ha (P0T1N2) significantly produced plants with the highest number of leaves per plant.

Table 11: *Interaction effects neem cake (N.) and Trichoderma species (T.) on the number of leaves at 5 and 7 weeks after planting (WAP) of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.*

Treatment combination	5 WAP	Treatment combination	7 WAP
(P×N)			
P0N0	7.22±0.22[g]	P0T0N0	10.33±0.33[fgh]
P1N0	6.44±0.34[h]	P0T1N0	11.33±0.33[def]
P2N0	6.33±0.4[h]	P0T2N0	10.67±0.33[efg]
P3N0	6.33±0.41[h]	P1T0N0	7.33±0.33[j]
P0N1	9.78±0.22[b]	P1T1N0	9.33±0.33[hi]
P1N1	8.11±0.26[f]	P1T2N0	8.67±0.33[i]
P2N1	8.22±0.43[ef]	P2T0N0	0.00±0.00[k]
P3N1	8.78±0.40[de]	P2T1N0	9.67±0.33[ghi]
P0N2	11.22±0.40[a]	P2T2N0	8.67±0.33[i]
P1N2	8.67±0.33[def]	P3T0N0	0.00±0.00[k]
P2N2	9.67±0.62[bc]	P3T1N0	9.33±0.33[hi]
P3N2	9.11±0.48[cd]	P3T2N0	8.67±0.33[i]
(P×T)		P0T0N1	11.67±0.67[de]
P0T0	8.89±0.46[cd]	P0T1N1	13.00±0.58[c]
P0T1	10.11±0.70[a]	P0T2N1	11.67±0.33[de]
P0T2	9.22±0.70[bc]	P1T0N1	9.33±0.33[hi]
P1T0	6.89±0.46[f]	P1T1N1	10.67±0.33[efg]
P1T1	8.56±0.38[d]	P1T2N1	10.33±0.33[fgh]
P1T2	7.78±0.32[e]	P2T0N1	8.67±0.33[i]
P2T0	6.22±0.43[g]	P2T1N1	11.33±0.33[def]
P2T1	9.44±0.56[b]	P2T2N1	10.67±0.33[efg]
P2T2	8.56±0.56[d]	P3T0N1	9.67±0.67[ghi]
P3T0	6.56±0.44[g]	P3T1N1	12.33±0.33[cd]
P3T1	8.78±0.40[d]	P3T2N1	11.67±0.33[de]
P3T2	8.89±0.59[d]	P0T0N2	12.33±0.67[cd]
		P0T1N2	17.67±0.33[a]
		P0T2N2	15.67±0.33[b]
		P1T0N2	10.67±0.33[efg]
		P1T1N2	11.67±0.33[de]
		P1T2N2	10.33±0.33[fgh]
		P2T0N2	9.33±0.33[hi]
		P2T1N2	13.33±0.33[c]
		P2T2N2	12.33±0.33[cd]
		P3T0N2	9.33±0.33[hi]
		P3T1N2	11.67±0.33[de]
		P3T2N2	10.33±0.33[fgh]

**Means within a column followed by the same letter of the alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at the 5% probability level.*
For key please see Table 1.

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Table 12: *Main effects of neem cake (N.) and Trichoderma species (T.) on the number of pods per plant and fresh pod weight (g) per plant of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.*

Neem (t/ha)	Number of pods per plant	Fresh pod weight (g) per plant
N0 0.00	2.83±0.26^{c}	16.53±1.39^{c}
N1 2.00	5.14±0.14^{b}	65.89±0.79^{b}
N2 4.00	6.31±0.20^{a}	80.21±1.79^{a}
***Trichoderma* species (T.)**		
T0 (control)	4.03±0.38^{c}	49.04±5.06^{c}
T1 *Trichoderma viride*	5.33±0.29^{a}	58.36±4.69^{a}
T2 *Trichoderma harzianum*	4.92±0.23^{b}	55.23± 4.54^{b}
Pathogens (P.)		
Po (control)	5.82±0.34^{a}	62.65±5.93^{a}
P1 *Meloidogyne incognita* alone	4.82±0.30^{b}	54.01±5.15^{b}
P2 *Sclerotium rolfsii* alone	4.67±0.40^{b}	53.57±5.67^{c}
P3 *M. incognita* + *S. rolfsii*	3.74± 0.31^{c}	46.62±5.10^{d}

** Means within a column followed by the same letter of the alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at the 5% probability level.*

Table 13: *Main effects of neem cake (N.) and Trichoderma species (T.) on the fresh root weight per plant, dry leaf weight per plant and dry stem weight per plant of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.*

Neem (t/ha)	Fresh root weight per plant	Dry leaf weight per plant	Dry stem weight per plant
N0 0.00	4.05±0.23^{c}	2.38± 0.21^{c}	2.23±0.21^{c}
N1 2.00	6.14±0.45^{b}	5.20±0.25^{b}	3.98±0.18^{b}
N2 4.00	7.42±0.53^{a}	7.17±0.38^{a}	6.17±0.51^{a}
***Trichoderma* Species (T.)**			
T0 (control)	4.72±0.40^{c}	3.20±0.32^{c}	3.17±0.38^{c}
T1 *Trichoderma viride*	7.13±0.53^{a}	6.24±0.43^{a}	4.86±0.46^{a}
T2 *Trichoderma harzianum*	5.76±0.42^{b}	5.30±0.39^{b}	4.36±0.41^{b}
Pathogens (P.)			
P0 (control)	9.29±0.64^{a}	6.45±0.59^{a}	6.74±0.65^{a}
P1 *Meloidogyne incognita* alone	5.35±0.31^{b}	4.68±0.44^{b}	3.54±0.28^{b}
P2 *Sclerotium rolfsii* alone	4.72±0.23^{c}	4.33±0.44^{c}	3.37±0.31^{b}
P3 *M. incognita* + *S. rolfsii*	4.12±0.24^{d}	4.21±0.42^{c}	2.86±0.25^{c}

** Means within a column followed by the same letter of the alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at the 5% probability level.*

Main effects of neem cake, Trichoderma species, and their interactions on the number of pods per plant and fresh pod weight (g) per plant inoculated with Meloidogyne incognita and Sclerotium rolfsii

Table 14 shows that inoculation of okra plants with pathogens significantly (P≤0.05) reduced the number of pods per plant. Combined inoculation of *M. incognita* and *S. rolfsii* produced okra plants with the lowest pods per plant. A significant increase in the number of pods per okra plant was obtained following soil amendment with 4.00 t/ha of neem. For *Trichoderma* species, *T. viride* - treated plants produced significantly (P≤0.05) more pods than *T. harzianum* - treated plants. Fresh pod weight per okra plants followed the trend of the number of pods per plant. Although inoculation of okra plants with pathogens had a significant (P≤0.05) decrease in fresh pod weight, application of neem cake and *Trichoderma* species significantly increased fresh pod weights. Plants amended with 4.00 t/ha of neem cake and treated with *T. viride* significantly increased fresh pod weight per plant compared to the control.

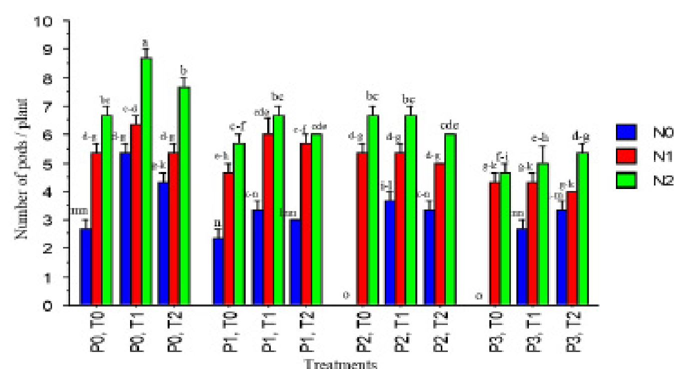

Figure 3: *Interaction effects of neem cake and Trichoderma species on the number of pods per plant.*
For key please see Figure 1.

Figure 3 shows that application of treatment combinations significantly (P≤0.05) led to increase in number of pods and fresh pod weight per okra plant. Okra plants with the highest number of pods per plant were obtained from soil amended with P0T1N2, followed by P0T2N2. Similarly, Figure 4 shows that fresh pod weight of okra plants with P0T1N2 soil amendment was significantly the highest, followed by P0T2N2.

Table 14: *Interaction effects of neem cake and Trichoderma species (T.) on the fresh root weight(g)/ okra plant and dry stem weight (g)/ plant of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii*

Treatment Combination	Fresh root weight per plant	Dry stem weight per Plant
P0T0N0	4.24±0.05^{op}	2.81±0.07^{l}
P0T1N0	6.73± 0.47^{f}	4.27±0.33^{h}
P0T2N0	5.22± 0.06^{j-m}	4.10± 0.34^{i}
P1T0N0	3.20±0.11^{q}	1.81±0.07^{q}
P1T1N0	4.35±0.27^{nop}	2.54± 0.15^{m-p}
P1T2N0	3.72± 0.18^{pq}	2.09±0.05^{opq}
P2T0N0	2.20± 0.03^{r}	0.00±0.00^{r}
P2T1N0	4.68± 0.29^{k-o}	2.63±0.13^{mno}
P2T2N0	4.30± 0.29^{op}	2.20±0.03^{n-q}
P3T0N0	1.57± 0.15^{r}	0.00±0.00^{r}
P3T1N0	4.65±0.38^{I-o}	2.31±0.08^{m-q}
P3T2N0	3.69±0.22^{pq}	1.93±0.03^{pq}
P0T0N1	8.49±0.37^{e}	5.08±0.47^{fg}
P0T1N1	12.14± 0.09^{b}	5.05±0.15^{de}
P0T2N1	10.48±0.38^{d}	5.56±0.18^{ef}
P1T0N1	4.84± 0.19^{k-o}	2.64±0.09^{mno}
P1T1N1	6.15±0.26^{fgh}	4.09±0.16^{i}
P1T2N1	5.10± 0.08^{j-n}	3.71±0.24^{ijk}
P2T0N1	3.75±0.19^{pq}	2.96±0.08^{lm}
P2T1N1	5.46± 0.13^{hijk}	4.10±0.08^{i}
P2T2N1	4.86±0.13^{k-o}	3.93±0.06^{ijk}
P3T0NI	3.13± 0.15^{q}	2.81±0.04^{lmn}
P3T1N1	5.22±0.06^{j-m}	3.67± 0.22^{ijk}
P3T2N1	4.11±0.07^{op}	3.33± 0.07^{kl}
P0T0N2	10.05±0.29^{d}	8.82±0.23^{c}
P0T1N2	14.84± 0.42^{a}	12.81±0.26^{a}
P0T2N2	11.45± 0.59^{c}	11.34±0.57^{b}
P1T0N2	5.67± 0.26^{ghij}	3.60±0.31^{ijk}
P1T1N2	8.80±0.22^{e}	6.23± 0.35^{d}
P1T2N2	6.30± 0.16^{fg}	5.17± 0.10^{fg}
P2T0N2	5.39± 0.13^{ijkl}	4.04± 0.14^{ij}
P2T1N2	6.55±0.17^{f}	5.57± 0.19^{ef}
P2T2N2	5.28±0.18^{i-m}	4.84±0.15^{gh}
P3T0N2	4.15± 0.10^{op}	3.40±0.21^{jkl}
P3T1N2	6.00±0.17^{fghi}	4.20±0.06^{i}
P3T2N2	4.55±0.04^{mno}	4.07±0.07^{i}

** Means within a column followed by the same letters of the alphabet are not significantly different from one another based on Duncan's New Multiple Range Test (DNMRT) at the 5% probability level.*
For key please see Table 1.

Main effects of neem cake, Trichoderma species, and their interactions on fresh root weight per plant, dry leaf weight per plant, and dry stem weight per plant of an okra plant inoculated with Meloidogyne incognita and Sclerotium rolfsii.

Table 14 shows that inoculation of okra plants with pathogens significantly (P≤0.05) reduced fresh root weight per plant, with combined inoculation of *M. incognita* and *S. rolfsii* having the least fresh root weight per plant. The introduction of neem cake treatment significantly (P≤0.05) increased fresh root weight per plant with the highest weight obtained when plants were treated with 4.00 t/ha of neem cake. *Trichoderma viride* treated plants produced okra plants with significantly (P≤0.05) higher fresh root weight, followed by *Trichoderma harzianum*. Dry leaf weight was also significantly (P≤0.05) reduced following inoculation with the pathogens. However, the application of neem cake significantly improved dry leaf weight per plant, with the highest dry leaf weight obtained from plants treated with 4.00 t/ha of neem cake. *T. viride* treated plants also had significantly (P≤0.05) higher dry leaf weight than *T. harzianum*.

Similar trends of results in dry leaf weight per plant were obtained for dry stem weight per plant where application of both neem cake and *Trichoderma* species significantly increased the dry stem weight of okra plants.

Table 14 and Figure 5, show that plants inoculated with *M. incognita* alone, *S. rolfsii* alone or *M. incognita* in combination with *S. rolfsii* without *Trichoderma* and neem cake (P1T0N0, P2T0N0 and P3T0N0) had significantly (P≤0.05) the lowest fresh roots weight per plant. Among all the treatment combinations, okra plants inoculated with *T. viride* and amended with 4.00 t/ha of neem cake without pathogens (P0T1N2) had significantly (P≤0.05) the highest fresh root weight per plant. Dry leaf weight per plant and dry stem weight per plant followed the trend of fresh root weight per plant. However, among all the treatment combinations, okra plants treated with *T. viride* and amended with 4.00 t/ha of neem cake without pathogens (P0T1N2) had significantly (P≤0.05) the highest dry leaf weight and dry stem weight per plant.

The results from this study show that there was a synergistic interaction between *M. incognita* and *S. rolfsii*.

The severity of root galling and basal stem rot on okra were higher when both pathogens were concomitantly inoculated relative to individual inoculation. Successive application of neem cake at a rate of 4.00 t/ha and *Trichoderma* species were very efficacious in ameliorating root galling and nematode infestation of okra plants. The high rate of galling observed in the okra plant may be attributed to the environment, especially considering that the soil used for the experiments had very high sand contents. Earlier reports stated that the activities of nematodes are enhanced in soil with high sand contents (Agu, 2008; Olewe, 2005).

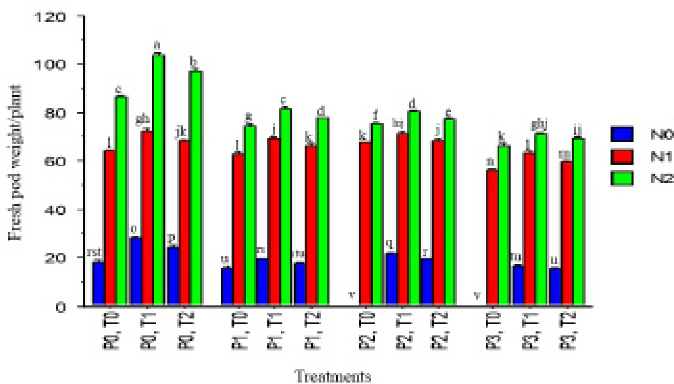

Figure 4: *Interaction effects of neem cake and Trichoderma species on the fresh pod weight per plant.*
For key please see Figure 1.

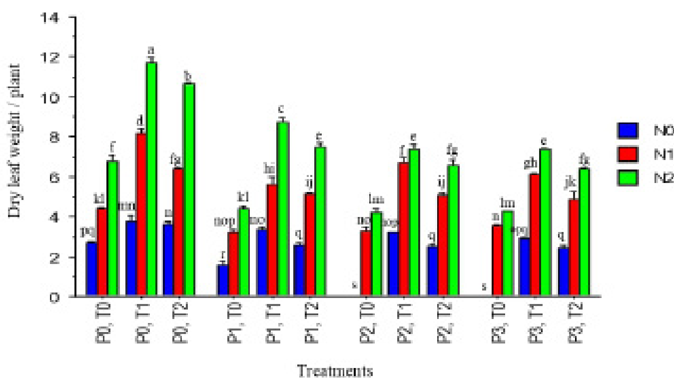

Figure 5: *Interaction effects of neem cake and Trichoderma species on the dry leaf weight/plant.*
For key please see Figure 1.

Among soil conditions, soil texture such as sand, silt, and clay particles, determine soil compactness and porosity thereby enhancing the availability of moisture and aeration for nematodes and it is considered as one of the most important soil characteristics related to nematode infestations in crops (Moore and Lawrence, 2013). Therefore, the high rate of galling and number of nematodes recorded per okra plant without soil amendment may not be unconnected with the nature of the soil used for this study, which was high in sand content. The results of this study are in conformity with Kim *et al.* (2016) who observed that root-knot galls were significantly higher in soils with 100% sand than in soils with a lower percentage of sand content.

From the observations made in the screenhouse experiment, it is possible that the application of neem cake and *Trichoderma* species was responsible for the reduction of galls and gall index in okra plants inoculated with *M. incognita*. It is possible that the two biocontrol agents must have released compounds with nematicidal effects into the soil to help reduce galls caused by *M. incognita*. Consequently, the gall index was reduced from highly susceptible (G.I = 5.00) in unamended soil to resistance (G.I = 2.00) in soils amended with neem cake and *Trichoderma* species. Similarly, the number of nematode per plant, nematode density in soil and nematode reproduction factor were significantly reduced following amendment of the soil with neem and *Trichoderma* species. These findings are in conformity with earlier reports by many authors that the application of neem compost and *Trichoderma* species solution were effective in reducing root galling, nematode population in root and soil caused by *M. incognita* thereby enhancing the growth of okra and cowpea (Atungwu *et al*., 2009; Chimbekujwo and Bukar, 2013, Radwan *et al*., 2012).

Earlier, Olabiyi *et al*. (2016) reported a significant reduction in nematode reproduction factor following soil amendment with neem compost and *T. harzianum*. Al-Hazmi and Tangjaveed (2015) also reported significant reduction in root galling, egg production, and soil juveniles following soil amendment with *T. harzianum* and *T. viride*. The neem cake was found to be more effective at a higher rate of 4.00 t/ha than 2.00 t/ha in reducing galls and nematode population in the soil. According to Akhatar and Mahmood (1995) neem cake contains phenols, tanins, flavonoids and a group of chemicals such as salanin, thironemonie and azadirachtin that are nematicidal at certain concentrations. This result corroborates the report that many neem preparations from seeds, leaves, oil cakes that serve as fertilizer, kernel oil, roots, and barks have shown nematicidal activity (Akhatar, 1996). This may suggest that at a higher concentration of 4.0 t/ha of neem cake, the nematicidal properties were high enough to effectively reduced root galls and nematode population than at a lower rate of 2.00 t/ha. The car-

bon to nitrogen (C: N) ratio is significant in composting because microorganisms need a good balance of carbon and nitrogen in order to remain active (Akratos et al., 2017). High C: N ratios can lead to prolonged composting duration and low C: N ratios enhance mineralization (Akratos et al., 2017).

According to Brust (2019), when an organic substrate has a C: N ratio of between 1-15, rapid mineralization and release of nitrogen occurs, which is available for plant uptake, and the lower the N: C, the more rapidly nitrogen will be released into the soil for immediate crop use (Watson et al., 2002). A carbon: nitrogen ratio greater than 35 results in microbial immobilization. However, the ratio of 20 – 30 results in equilibrium state. Therefore, the ratio of C: N obtained from the neem cake (13.88) suggests quick mineralization and enhancement of nematicidal compounds from the treatment, which helped in reducing root galling caused by *M. incognita* infestation. A similar report was made by Akhatar and mahmood (1995) where application of composted manure, neem oil cakes, and castor with a low C: N ratio (6 - 10) and high ammonium nitrogen content was observed to decrease plant parasites and nematode population in soil. The release of nematicidal compounds in soil such, as ammonia, organic acid during degradation and changes in soil physiochemical properties have been reported to be involved in nematode suppression. It is very likely that the C: N ratio of neem cake in this study must have enhanced faster decomposting and release of nematicidal compounds into the soil for suppression of nematode activities. This result substantiates earlier reports that many extract from the neem plant such as seeds, leaves, root, and barks, have nematicidal activities (Akhatar, 2000; Yasmin et al., 2003; Renco, 2013; Umana et al., 2014).

Numerous secondary metabolites produced by *Trichoderma* species could directly or indirectly inhibit the growth of several plant pathogens. Regarding the efficacy of *Trichoderma* species observed in this study, *T. viride* was more effective in inhibiting root galling, nematode population, and nematode reproduction factor (R_f). This may be credited to the faster growth and movement of *T. viride* in culture media, as reported by Shah *et al.* (2012). In their research, they found out that *T. viride* grow faster in different cultural media than *T. harzianum* and *T. pseudokoningii*. It is likely that the *T. viride* may utilize nutrients faster in the soil to develop the necessary mechanisms and pathways required to attack and suppressed *M. incognita* and its related galling and nematode reproduction than *T. harzianum*.

The interaction between neem and *Trichoderma* species was significant in reducing galling and nematode population, compared with the control. In all cases, the interaction of neem cake at the rate of 4.00 t/ha with *T. viride* had the most effective interaction effects on root galling and nematode population. The bioactive compounds in neem cake such as metriantriol, nimbidin, azadirachtin, nimbin, azadirone and solanin at a higher concentration must have interacted more efficiently with *T. viride* than *T. harzianum,* thereby resulting in significantly lower number of galls and number of nematodes per okra plant inoculated with *M. incognita*.

According to Part and Pandey (2002) a better growth of chickpea plant and reduction of root-knot nematode following interaction of neem cake and *Trichoderma* species may be due to additional supply of nutrients available from organic substance and production of toxin which which was lethal to root-knot nematode. It is also possible that the nutrients released by the neem cake in combination with *Trichoderma* species must have provided a favourable environment to enhance other soil antagonists that could helped reduce the damage potentials of soil nematodes in this study.

S. rolfsii is a necrotrophic soil borne pathogen that produces abundance of white mycelium and brown sclerotia in culture media and on infected plants (Aycock, 1966). The *S. rolfsii* root colonization index of okra plant was significant following inoculation with *Sclerotium rolfsii*. Neem cake at 4.00 t/ha was more efficacious in reducing the *S. rolfsii* root colonization index. According to Narain and Satapathy (2011) plant extracts such as *Tinospora cardifolia*, *Azadirachta indica*, *Datura, Canabis*, *Thiya,* and Eucalyptus can be used as bio-control agents against *S. rolfsii*. However, among all the tested plant extracts *Azadirachta indica* (neem) extract was found by the authors to offer the best control effect at higher concentrations on *Sclerotium rolfsii*. This findings of the present study is in corroboration with several reports by authors that neem cake extract is very effective in suppressing *S. rolfsii* root colonization index (Tang *et al*., 2015; Guerra *et al*., 2015; Sab *et al*, 2014; Dania *et al*., 2014; Butt, 2016). There was also significant reduction in *S. rolfsii* root colonization index following the application

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of *Trichoderma* species, with *T. viride* having a higher suppressing efficacy than *T. harzianum*. *Trichoderma* species inhibits *S. rolfsii* by releasing enzymes such as chitanases, glucanases, and proteinases (Biswas and Sen, 2000). For *T. viride* to have a higher suppressing effect on *S. rolfsii* root colonization index, it is possible that it produced these chemicals in a higher quantity and at a faster rate than *T. harzianum*. A study by Abd-Allah (2005) showed that *Trichoderma* species was better in control of *S. rolfsii* than *Bacillus subtilis*. The results on the efficacy of *Trichoderma* species in controlling *S. rolfsii* root colonization index is in conformity with Radwan *et al*., (2006) that *T. harzianum* and *T. viride* were most effective against *S. rolfsii* and inhibited the growth by 92%.

In this study, there were significant increase in growth and yield of okra plants in soils amended with neem cake or inoculated with *Trichoderma* species compared to the unamended controls. Neem cake treated crops had increased height, especially at 4.00 t/ha at 3, 5, and 7 weeks after planting. As it has been advocated that neem cake enhances the nutrient contents of soil, the observed increase in plant height must have been enhanced by the release of nutrients from neem cake. This result is in conformity with Krishnaray *et al*. (2018) and Madhuri *et al*. (2021), who found that neem cake increased plant height of tomatoes. Yield indices are important agronomic traits of economic value. In this study, fresh root weight, dry leaf weight, and dry stem weight, number of pods, and fresh pod weights per plant were considered. Plant biomass, including root, leaf, and stem weights, is an important factor that affects the final yield of crops. Roots are responsible for the uptake of water and dissolve minerals from the soil and can also act as food storage facilities in some plants. The stem connects the roots to the branches and the leaves, and the leaves contains the chlorophyll that necessitates food production by light absorbance; therefore, these parameters are very important in determining the final yield of crops. In this study, the fresh root, dry leaf, and dry stem weights of okra plants were higher in soil amended with neem and *Trichoderma* species compared to unamended pots, except for the fresh root weight, which was higher in plants grown in unamened soil than soils amended with *Trichoderma*.

Conclusion and Recommendations

A comparative study revealed there was a synergistic interaction between *M. incognita* and *S. rolfsii*. The severity of root galling and basal stem root was higher when both pathogens were concomitantly inoculated relative to individual inoculation. Amendment of soil with neem cake and *Trichoderma* species were effective in controlling root-knot nematodes, fungal activities, and improving the growth and yield of okra plant. Soil amended with neem cake at 4.00 t/ha was more effective in enhancing okra performance than at the rate of 2.00 t/ha, *T. viride* was a better enhancer of okra agronomic traits. Amendment of soil with neem cake and its combination with different inoculum loads of *Trichoderma* species significantly enhanced okra plant performance as a result of reduction in root galling.

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Novelty Statement

This study has demonstrated the possibility of using indigenous isolates of Trichoderma species in combination with neem cake in the management of Meloidogyne incognita and Sclerotium rolfsii in Screenhouse trial.

Author's Contribution

DE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of manuscript. IU, EJ and NE managed the analysis of study and 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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