Research Article

Management of *Meloidogyne incognita* (Kofoid & White) Chitwood and *Sclerotium rolfsii* Sacc. on Common Bean (*Phaseolus vulgaris* L.) in Field, Using *Trichoderma* species and Neem Cake

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Abstract |Common bean cultivation faces challenges due to notable pathogenic organisms. Nematode and fungal infestations are known to hinder production maximization. This research evaluated the effectiveness of two *Trichoderma species* alongside neem cake for controlling *Meloidogyne incognita* and *Sclerotium rolfsii*. Treatments consisted one gram of *Trichoderma viride* (2.40×10^7 CFU/g) and *Trichoderma harzianum* (1.40×10^7 CFU/g) applied together with neem cake at rates of 2.00 and 4.00 t/ha on experimental plots of common bean (*Phaseolus vulgaris*). Plots without inoculation or amendments acted as the control. A randomized complete block design with three replicates were used for the trial. Natural infestations of *M. incognita* and *S. rolfsii* were observed in soil with two-week-old common bean plants. Results revealed high susceptibility of common bean plants to *M. incognita* as well as *S. rolfsii* in absence of neem cake and *Trichoderma* species treatments. Modification using neem cake at 4.00 t/ha alongside *T. viride* lowered significantly ($P\leq0.05$) gall formation and population of nematodes. Soil inoculation with *Trichoderma* species considerably decreased root galling and nematode populations, with *T. viride* exhibiting superior efficacy compared to *T. harzianum*. Prevalence and severity of *S. rolfsii* infection were significantly ($P\leq0.05$) reduced in common bean plants that received both biocontrol agents relative to control. Growth and yield parameters were significantly ($P\leq0.05$) improved in common bean plants panted in neem cake modified soil and inoculated with *Trichoderma* species. The highest pod yield (21.08 t/ha) was recorded in plots that received *T. viride* combined with 4.00 t/ha of neem cake.

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Introduction

Phaseolus vulgaris L. is an herbaceous annual that is widely cultivated worldwide, including regions within Sub-Saharan Africa. The species (*P. vulgaris*) flourishes in temperate and subtropical environments, including areas in Africa, Central America, and South America. Its primary mode of cultivation pertains to the production of dry seeds, and this agricultural practice reveals a broad spectrum of variations in seed coat pigmentation and pod attributes. Dry legumes are acknowledged for their comprehensive nutrient profiles and phytochemical constituents, these are pivotal especially in connection with the role of legumes in reducing the risk of various health disorders (Camara *et al*., 2013). Indeed, several investigations have corroborated the effectiveness of common bean in the management of diabetes (Laleye *et al*., 2015) and obesity (Carai *et al*., 2009; Rondanelli *et al*., 2011). According to the Missouri Botanical Garden (2023), the most effective propagation strategy for this species involves sowing seeds in consistently moist, fertile, organically-rich, well-drained loam soil, subjected to full sunlight exposure.

P. vulgaris exhibits vulnerability to numerous pathogens that considerably diminish its agronomic productivity. The nematodes *M. hapla, M. javanica, M. incognita*, and the fungus *S. rolfsii* have been recognized as significant agents contributing to the infection of the common bean, resulting in marked reductions in growth and yield (Saka, 1990; Paparus *et al*., 2020). Plant-parasitic nematodes, which exhibit widespread distribution and possess a high fecundity rate, present considerable challenges in mitigating their detrimental effects on the crop, leading to extensive damage and yield losses (Luc *et al*., 2005). These nematodes target different parts of host plants, inducing symptoms such as wilting, chlorosis, stunted development, and gall formation in plant root system.

Likewise, *Sclerotium rolfsii* exhibits a broad host range, which includes potato, groundnut, soybean, sunflower, chili, tomato, *P. vulgaris*, cotton, lucerne, wheat, and onion, and it possesses the capacity to induce significant reductions in crop yields (Buensanteai *et al*., 2012). Managing *S. rolfsii* poses considerable difficulties in the absence of synthetic fungicides. Nevertheless, the application of synthetic pesticides not only exacerbates environmental degradation but also imposes a financial strain on rural agriculturalists in numerous developing nations, owing to the rising costs associated with these chemical agents (Bell, 2013). Recently, there has been a conspicuous transition from environmentally detrimental pesticides towards plant derived alternatives for pathogen management (Bassey *et al*., 2023). For example, the utilization of fungi such as *T. harzianum* and *T. viride* has become increasingly favored, alongside the development of additional plant-based pesticides aimed at controlling plant-parasitic nematodes, with the objective of mitigating the complications linked to synthetic nematicides (Bell, 2013). This present research evaluated the efficacy of two *Trichoderma species*, and Neem Cake (NC) in the control of *M. incognita* and *S. rolfsii*.

Materials and Methods

Experimental site and source of materials

The study was performed at Nyahasang area in Calabar municipality, located in Cross River State, located at 4.99° N latitude, 8.35° E longitude. Common bean seeds (*P. vulgaris*) were sourced at the Ministry of Agriculture, Cross River state, Nigeria. A stock culture of *T. viride* and *T. harzianum* was purchased in Department of Botany at the University of Calabar. The byproduct obtained during neem oil extraction, called NC was acquired from the Institute of Agricultural Research (IAR) in Zaria, Nigeria.

Sub culturing and spore count of trichoderma species

Potato Dextrose Agar (PDA) served as the culture medium for the sub-culturing of pure stock cultures of the two *Trichoderma* species. To quantify colony-forming units (CFU/g), the *T. viride* and *T. harzianum* were scraped and 1 gram of each species was accurately measured using an Ohaus weighing balance. The substrates measured was then placed in a beaker containing distilled water of 10 ml. This was subsequently mixed in a vortex mixer to achieve a homogeneous solution. A volume of 1ml from the diluted samples was pipetted into the hemacytometer for counting under a binocular microscope. The spore quantification of *Trichoderma* species followed the methodology outlined by Pitt and Poole (1981). The spore density was calculated as millions of CFU per gram of millet substrate, resultant spore numbers were utilized as inoculum loads. The quantified spore load for *T. viride* was 2.40×10^7 CFU/g of millet, while *T. harzianum* recorded a spore count of 1.40×10^7 CFU/g of millet.

Preparation of carrier for Trichoderma species

The method described by Sivan *et al*. (1984) was followed in the formulation of a substrate for *Trichoderma spp*. A sensitive weighing balance was utilized to measure 200g of millet grains, which were subsequently soaked in distilled water for a duration of 24 hours to facilitate fermentation prior to utilization. The grains underwent sterilization via autoclaving at 121°C for 15 minutes in a Bema bottle, and allowed to cool. Decontaminated cork borers containing mycelial discs from various *T. viride* and *T. harzianum* cultivated on PDA as pure cultures were introduced to the substrates. The substrates were incubated at ambient laboratory temperature for a period of 3 to 5 days prior to their deployment in the field for inoculation.

Application of neem cake (NC), planting of common bean seeds and inoculation of Trichoderma species

The application of NC was done at rates of 0.96 and 1.92 kg per plot, corresponding to 2 and 4 t/ha, respectively, according to the experimental design, two weeks preceding the planting; the control plots received no NC application. Three seeds of common bean were planted at a depth of 3 cm with an inter-row spacing of 50 cm and intra-row spacing of 30 cm, which were subsequently thinned to a single plant stand two weeks post-sowing, resulting in a density of 28 plants in each plot (66,667 plants/ha). One gram (1g) of various *Trichoderma* species was utilized for inoculation on the treatment plots, with uninoculated plots serving as the control.

Experimental design, data collection and statistical analysis

The experimental framework was established as a 3 x 3 factorial in a randomized complete block design (RCBD), replicated thrice, culminating in 9 treatment combinations and 27 experimental units or plots. Data were systematically gathered at biweekly intervals commencing three weeks post-sowing (3WAP) from eight central rows of plants. The parameters measured included: galls per plant (GPP), Mean Gall Index (MGI), root-knot disease incidence (RDI), nematode number per plant, nematode density in 250 grams of soil, incidence (%) of *S. rolfsii* disease and its severity index (SI), height of plants (cm), number of leaves, weight of fresh roots (g) per plant, number of pods per plant, yield of pods (t/ha), and dry weights of leaves (g) and stems (g) per plant. The acquired data were analyzed using analysis of variance (ANOVA), and significant means were evaluated through Duncan's New Multiple Range Test (DNMRT) at a 5% level of probability.

Results

Effects of neem cake (NC), Trichoderma species and their interactions on number of galls in each plant and MGI of common bean plants naturally infested with M. incognita and S. rolfsii.

Table 1 indicates that the quantity of GPP were higher significantly ($P \leq 0.05$) in common bean specimens cultivated in unamended soils devoid of NC or *Trichoderma* species. A dosage increase of NC caused a significant ($P \leq 0.05$) reduction in root galling. Plants exhibiting the lowest gall count were obtained from soil enriched with 4.00 t/ha of NC. The introduction of both biocontrol agents led to significant ($P \leq 0.05$) decrease in galls, in each plant when compared to the control group.

Table 1: *Effects of neem cake (N.), Trichoderma species (T.) and their interactions number of galls per plant and mean gall index of common bean plant naturally infested with M. incognita and S. rolfsii.*

Neem (t/ha)	Number of galls per plant	Mean gall index
N00.00	66.00 ± 8.90^{a}	3.26 ± 0.19^{a}
N12.00	50.33 ± 8.04^{b}	2.75 ± 0.16^{b}
N24.00	37.22 ± 5.93^{c}	2.18 ± 0.17^{c}
Trichoderma **species (T.)**		
T0 (control)	80.89 ± 6.11^{a}	3.21 ± 0.23^{a}
T1 *Trichoderma viride*	32.11 ± 4.02^{c}	2.33 ± 0.20^{b}
T2 *Trichoderma harzianum*	$40.56+2.74^{b}$	2.65 ± 0.15^{b}
Trichoderma **species and neem cake (Interactions)**		
T0N0		3.96 ± 0.21^{a}
T0N1		3.00 ± 0.29^{a}
T0N2		2.67 ± 0.17^{a}
T1N0		3.00 ± 0.00^{a}
T1N1		2.25 ± 0.14^{a}
T1N2		1.75 ± 0.25^{a}
T2N0		2.83 ± 0.17^{a}
T2N1		3.00 ± 0.00^{a}
T2N2		2.13 ± 0.19^{a}

Nevertheless, minimal gall count was recorded with the application of *T. viride*. In untreated plants, the average gall index was equally higher significantly ($P \leq 0.05$). The incorporation of NC into the soil markedly decreased the MGI, particularly at 4.00 t/ha. The MGI was significantly comparable in common bean plants subjected to both species of *Trichoderma*.

The interactive effects of *Trichoderma* species, as well as NC were significant ($P \leq 0.05$) on number of galls in each plant, as depicted in (Figure 1). The highest gall count was recorded in plants that did not receive either *Trichoderma* species or NC. Conversely, the combined application of treatments reduced significantly ($P \leq 0.05$) the GPP, resulting in the lowest counts.

Conversely, the combined application of treatments significantly (P≤0.05) lowered the number of galls in each plant, resulting in the lowest counts.

Neem cake and Trichoderma species interactive effects on RDI (%), on nematodes per plant as well as density of nematodes in common bean naturally infested with M. incognita and S. rolfsii

Table 2 shows that the utilization on treatment using NC reduces significantly (P≤0.05) incidence of root-knot disease at an application rate of 4.00 t/

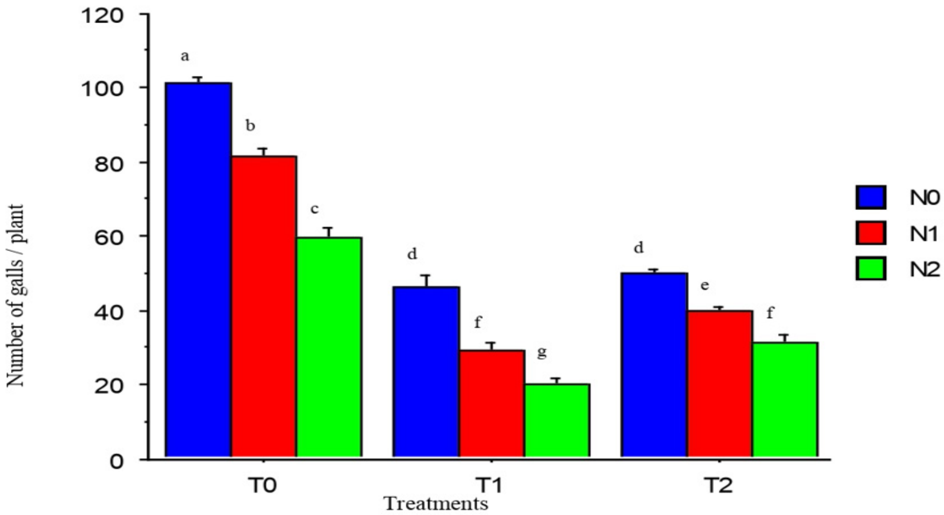

Figure 1: *Interaction effects of Trichoderma species in combination with neem cake on number of galls of M. incognita common bean plant in factorial field experiment. a = Significantly (P≥0.05) highest number of M. incognita gall in common bean plant (ANOVA and significant means were compared using duncan new multiple range test at 5% probability level)*

Table 2: *Effects of neem cake (N.), Trichoderma species (T.) and their interactions on root-knot disease incidence (%), number of nematodes per plant and nematode density in 250 grams of soil of common bean plant naturally infested with M. incognita and S. rolfsii.*

Neem (t/ha)	Root-knot disease incidence (%)	Number of nematodes per plant	Nematode density in 250 of soil
N0 0.00	75.00±2.08^a	3356.00±697.92^a	339.40±57.90^a
N12.00	70.83±3.61^a	1806.00±406.64^b	237.90±36.46^b
N24.00	61.10±3.26^b	1118.00+286.73^c	161.00±33.71^c
Trichoderma **species (T.)**			
T0 (control)	73.60±3.26^a	3936.00±577.50^a	416.20±40.52^a
T1 *Trichoderma viride*	65.30±4.05^a	1032.00±213.54^c	151.00±17.25^c
T2 *Trichoderma harzianum*	68.10±3.03^a	1312.00±206.33^b	171.10±20.76^b
Trichoderma **species and neem cake(Interactions)**			
T0N0	79.20±4.17^a		570.00±5.77^a
T0N1	75.00±7.22^a		383.00±4.04^b
T0N2	66.70± 4.17^a		295.70±3.18^c
T1N0	75.00± 0.00^a		207.70±5.04^e
T1N1	62.50±7.22^a		156.00±4.93^g
T1N2	58.30±8.33^a		89.30±1.45^h
T2N0	70.80±4.17^a		240.70±5.78^d
T2N1	75.00± 0.00^a		174.70±5.93^f
T2N2	58.30± 4.17^a		98.00±2.31^h

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

ha, whereas the application of 2.00 t/ha NC showed statistical significance (P≤0.05) similar to the control. The application of the two *T. viride* and *T. harzianum* did not produce significant (P≥0.05) effects regarding diseases of root-knot incidence on common beans. The quantity of nematodes per plant was reduced significantly (P≤0.05) with NC application. The most substantial reduction was observed at the application rate of 4.00 t/ha. The application of *Trichoderma* species led to a significant (P≤0.05) reduction in nematodes per plant when compared to control (Figure 2). Plants treated using *T. viride* exhibited a significantly lowered the number of nematodes in each plant, in contrast to the ones treated using *T. harzianum*. The concentration of nematodes in the soil paralleled the trend observed in the number of nematodes per plant. Soil enhanced with 4.00 t/ha of NC significantly decreased the nematode density in the soil. The application of *T. viride* and *T. harzianum* also significantly reduced the density of nematode in the soil. Nevertheless, plants inoculated using *T. viride* revealed smallest nematode density in the soil.

The interaction effects of *T. viride* and *T. harzianum,* as well as NC did not yield significant (P≥0.05) effects on the disease incidence of root-knot in common bean plants. The interaction effects of the treatment applications reduce significantly (P≤0.05) the number of nematodes per plant and nematode density in the soil compared to the controls. However, the interactive effect of NC at 4.00 t/ha alongside *T. viride* resulted in lowered nematodes number in each plant, as well as density of nematode in the soil, in comparison to the control.

Interactive effect of neem cake, Trichoderma species on S. rolfsii disease incidence and SI of common bean infested with M. incognita and S. rolfsii

Table 3 illustrated that NC as well as *Trichoderma* species utilization diminishes significantly (P≤0.05) incidence of *S. rolfsii* disease in common bean plants. Specifically, plants treated with NC 2.00 t/ha rate exhibited significantly small (P≤0.05) incidence of *S. rolfsii* when compared those treated 4.00 t/ha. Regarding the *Trichoderma* species, common bean plants cultivated in soil enriched with *T. harzianum* demonstrated a significantly (P≤0.05) lower incidence of *S. rolfsii* than those that received *T. viride.* The patterns observed in the *S. rolfsii* disease SI for common bean plants were congruent with the findings on disease incidence, where the plants inoculated using 2.00 t/ha of NC and *T. harzianum* displayed reduction significantly (P≤0.05) regarding severity of *S. rolfsii* infection.

Table 3: *Effects of neem cake (N.) Trichoderma species (T.) and their interactions on Sclerotium rolfsii disease incidence and Sclerotium rolfsii disease severity index of common bean plant naturally infested with M. incognita and S. rolfsii.*

Neem (t/ha)	S. Rolfsii disease incidence	S. Rolfsii disease severity index
N00.00	52.41 (46.66)± 8.28[a]	37.82 (37.86)±2.93[a]
N12.00	29.37 (26.15)±4.37[c]	22.49 (28.22)±1.38[c]
N24.00	21.43 (31.45)±3.86[b]	26.39 (30.81)±1.78[b]
Trichoderma **species (T.)**		
T0 (control)	55.16 (47.03)±7.65[a]	36.37 (36.96)±3.20[a]
T1 *Trichoderma viride*	20.27 (32.38)±3.32[b]	27.31 (31.38)±2.17[b]
T2 *Trichoderma harzianum*	27.78 (26.85)±3.66[c]	23.02 (28.55)±1.73[c]
Trichoderma **species and neem cake (interactions)**		
T0N0	84.52 (67.04)±3.15[a]	48.79 (44.30)± 0.92[a]
T0N1	45.24 (34.58)±4.29[cd]	27.78 (31.80)±0.40[de]
T0N2	35.71 (39.46)±2.06[bc]	32.54 (34.78)±0.52[bc]
T1N0	32.24 (42.25)±1.98[b]	34.92 (36.21)±2.18[b]
T1N1	17.86 (24.93)±2.06[e]	21.03 (27.29)±0.20[g]
T1N2	10.71 (29.96)±2.06[de]	25.99 (30.64)±1.43[f]
T2N0	40.48 (36.69)±4.29[bc]	29.76 (33.06)±0.60[cd]
T2N1	25.00 (18.94)±2.06[f]	18.65 (25.58)±0.40[g]
T2N2	17.86 (24.93)±2.06[e]	20.63 (27.01)±0.53[g]

The interactive effects of *T. viride* and *T. harzianum* as well as NC regarding both the incidence as well as severity of *S. rolfsii* disease indicated that common bean plants devoid of both *T. viride* and *T. harzianum* and NC exhibited the highest levels of disease incidence and severity, which were statistically significant (P≤0.05). Conversely, the combined application of both treatments resulted to a reduction significantly (P≤0.05) of incidence and severity of *S. rolfsii* disease in common bean plants.

Neem cake (NC) and Trichoderma species interactive effects on height of common beans, naturally infested with M. incognita and S. rolfsii at 3, 5 and 7 at the end of planting

Table 4 indicates that NC application rate of 4.00 t/ha enhanced plant height significantly (P≤0.05) at three weeks post-sowing when compared to a rate of 2.00 t/ha and the control. *T. viride* and *T. harzianum* application did not create any statistically significant (P≥0.05) impact on plant height at three weeks post-sowing. At five and seven weeks after planting, soil amended with 4.00 t/ha of NC demonstrated a greater height significantly (P≤0.05), followed by 2.00 t/ha in comparison to the control. Soil inoculated with both *T. viride* and *T. harzianum* had similar heights at five weeks after planting. At seven weeks after planting, the inoculation of soil with both *T. viride* and *T. harzianum* caused an improvement in plant height, with the optimal height achieved when *T. viride* was applied. There were no statistically significant (P≥0.05) effects on plant height at three weeks after planting. Nevertheless, at five and seven weeks following planting, the tallest plants were observed in soil treated with (T1N2 and T2N2). Overall, plant height exhibited an increase as the duration of weeks progressed.

Neem cake (NC) and Trichoderma species interactive effects of leaves of common beans, naturally infested with M. incognita and S. rolfsii at 3, 5 and 7 at the end of planting.

Soil modified using 4.00 t/ha (Table 5) of NC increased leaf production significantly (P≤0.05) in common bean, followed by 2.00 t/ha in comparison to the control. The application of *Trichoderma* species did not result in any significant improvement on the number of leaves in each plant at three weeks post-sowing. At five- and seven-weeks post-sowing, soil 4.00 t/ha of NC significantly (P≤0.05) promoted leaf production in common bean plants, followed by 2.00 t/ha compared to the control. The application

Table 4: *Effects of neem cake (N.), Trichoderma species (T.) and their interactions on plant height (cm) of common bean plant naturally infested with M. incognita and S. rolfsii.*

Neem (t/ha)	3 Weeks after planting (WAP)	5WAP	7WAP
N00.00	8.84±0.13[c]	21.86±0.83[c]	39.24±0.97[c]
N12.00	10.58±0.14[a]	24.53±0.67[b]	45.63±0.63[b]
N24.00	12.00±0.11[a]	26.69±0.63[a]	49.64±0.85[a]
Trichoderma **species (T.)**			
T0 (control)	10.26±0.47[a]	21.57±0.83[b]	41.98±1.71[c]
T1 *Trichoderma viride*	10.60±0.48[a]	25.79±0.73[a]	46.97±1.70[a]
T2 *Trichoderma harzianum*	10.55±0.45[a]	25.71±0.57[a]	45.57±1.21[b]
Trichoderma **species and neem cake (Interactions)**			
T0N0	8.64±0.16[a]	18.59±0.33[f]	35.46±0.54[g]
T0N1	10.28±0.10[a]	21.92±0.30[e]	43.58±0.44[e]
T0N2	11.86±0.21[a]	24.22±0.22[c]	46.88±0.46[cd]
T1N0	8.84±0.15[a]	23.25±0.26[d]	40.92±0.38[f]
T1N1	10.85±0.20[a]	25.93±0.34[b]	47.50±0.72[c]
T1N2	12.12±0.14[a]	28.20±0.19[a]	52.48±0.52[a]
T2N0	9.04±0.33[a]	23.75±0.14[cd]	41.34±0.44[f]
T2N1	10.60±0.31[a]	25.73± 0.22[b]	45.80±0.33[d]
T2N2	12.01 ±0.24[a]	27.65± 0.26[a]	49.57±0.59[b]

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

of both *Trichoderma* species at five- and seven-weeks post-sowing demonstrated statistically similar leaf production. *Trichoderma* species and NC showed no significant (P≥0.05) improvements on the leaves in each plant at three weeks post-sowing. Nevertheless, at five and seven weeks after planting, the interaction effects significantly (P≤0.05) resulted in highest leaf production in soils treated with (T2N2), next to T1N2.

Neem cake (NC) and Trichoderma species interactive effects on fresh root weight per plant, dry leaf weight per plant and dry stem weight per plant of common beans naturally infested with M. incognita and S. rolfsii.

Table 6 elucidates that significant effects was seen on *Trichoderma*. The most pronounced fresh root weight per plant was recorded in common bean plants that received 4.00 t/ha of NC, followed by 2.00 t/ha

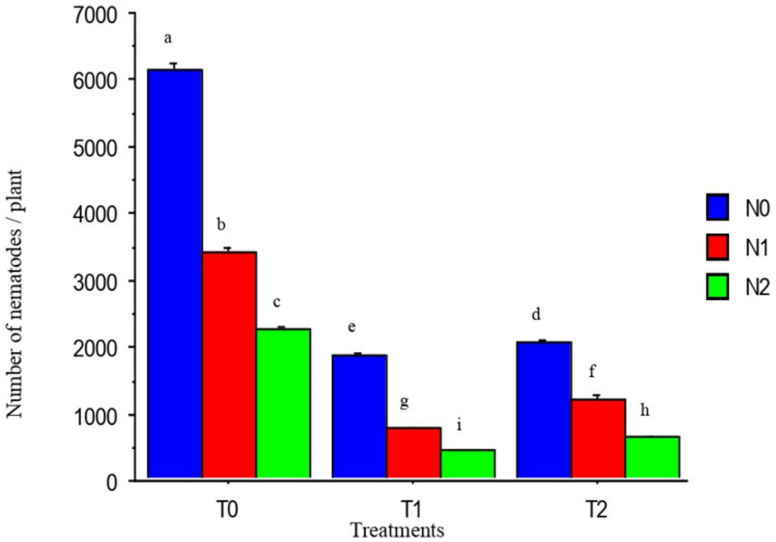

Figure 2: *Interaction effects of Trichoderma species in combination with neem cake on number of nematodes/ common bean plant in factorial field experiment. a = Significantly (P≥0.05) highest number of M. incognita gall in common bean plant (ANOVA and significant means were compared using duncan new multiple range test at 5% probability level)*

Table 5: *Effect of neem cake (N.), Trichoderma species (T.) and their interactions on number of leaves of common bean plant naturally infested with M. incognita and S. rolfsii.*

Neem (t/ha)	**3WAP**	**5WAP**	**7WAP**
N00.00	5.22±0.15[c]	9.67±0.33[c]	11.67±0.44[c]
N12.00	6.11±0.11[b]	11.44±0.34[b]	13.44± 0.34[b]
N24.00	6.67±0.17[a]	13.44±0.44[a]	14.89±0.59[a]
Trichoderma **species (T.)**			
T0 (control)	5.89±0.20[a]	10.33±0.50[b]	11.89±0.35[b]
T1 *Trichoderma viride*	6.11±0.31[a]	12.33±0.78[a]	13.78±0.76[a]
T2 *Trichoderma harzianum*	6.00± 0.24[a]	11.89± 0.42[a]	14.33±0.44[a]
Trichoderma **species and neem cake (Interactions)**			
T0N0	5.33±0.33[a]	8.67±0.33[f]	10.67±0.33[f]
T0N1	6.00±0.00[a]	10.33±0.33[e]	12.33±0.33[de]
T0N2	6.33±0.33[a]	12.00±0.00[c]	12.67±0.33[cd]
T1N0	5.00±0.00[a]	9.67±0.33[e]	11.33±0.67[ef]
T1N1	6.33±0.33[a]	12.33±0.33[c]	13.67±0.33[bc]
T1N2	7.00±0.00[a]	15.00±0.00[a]	16.33±0.33[a]
T2N0	5.33±0.33[a]	10.67±0.33[de]	13.00±0.58[cd]
T2N1	6.00±0.00[a]	11.67±0.33[d]	14.33±0.33[b]
T2N2	6.67±0.33[a]	13.33±0.33[b]	15.67±0.33[a]

compared to control group. Plants that were not treated with *Trichoderma* species exhibited greater fresh root weight when compared to plants treated with the biocontrol agents. The dry leaf weight in each plant demonstrated significant (P≤0.05) variations in common bean plants treated with different rates of NC and *Trichoderma* spp. The highest dry leaf weight per plant was achieved when the soil was modified with 4.00 t/ha of NC. Conversely, *T. viride* yielded a dry leaf that was significantly (P≤0.05) compared to the control. The dry stem weight per plant exhibited a trend consistent with that of the dry leaf weight per plant. Soil enriched with 4.00 t/ha of NC exhibited the highest dry weight per plant, followed by the 2.00 t/ha application. Regarding *Trichoderma* spp., *T. viride* manifested significantly (P≤0.05) highest dry stem weight per plant.

These interactive effects were significant (P≤0.05) regarding fresh root weight in each plant. The maximum fresh root weight per plant was attained by T0N0, followed closely by T1N2. The interactive effect was significant (P≤0.05) concerning dry leaf weight of each plant. The greatest, yet statistically similar dry leaf weights were achieved by common bean plants treated with T0N2, T1N1, T1N2, and T2N2. Furthermore, the interactive effects on dry stem weight were equally significant (P≤0.05). Nevertheless, greatest dry stem weight was obtained from plants in T1N2 treatment group.

Neem cake (NC) and Trichoderma species interactive effects on number of pods per plant and pod yield t/ha of common beans naturally infested with M. incognita and S. rolfsii

Table 7 and Figure 3 revealed the number of pods in each plant were significant (P≤0.05) in NC, as well as *Trichoderma* species enriched plants, when put side by side with control group. A greater pods per plant numbers were observed when the soil was amended using 4.00 t/ha of NC, in comparison to the control. Plots inoculated with either species of *Trichoderma* exhibited a pod count that was significantly (P≤0.05) greater with reference to the control. The pod yield was equally significant (P≤0.05) in plants cultivated in soil modified with 4.00 t/ha of NC. Soil amended with 4.00 t/ha of NC significantly yielded the highest pod yield, followed by 2.00 t/ha, when compared to the control. Both biocontrol agents significantly (P≤0.05) yielded greatest pod production relative to control, with plants treated with *T. viride* demonstrating the

Table 6: *Effects neem cake (N.), Trichoderma species (T.) and their interactions on fresh root weight per plant, dry leaf weight per plant and dry stem weight per plant of common bean naturally infested with M. incognita and S. rolfsii.*

Neem (t/ha)	Fresh root weight per plant	Dry leaf weight per plant	Dry stem weight per plant
N00.00	27.47±1.94[c]	33.74±2.93[c]	134.10±12.66[c]
N12.00	30.28±0.68[b]	43.55±1.67[b]	189.80±14.43[b]
N24.00	31.32±0.49[a]	48.53±1.09[a]	211.90±17.09[a]
Trichoderma **species (T.)**			
To (control)	32.63+0.72[a]	35.64+3.61[c]	121.20+9.47[c]
T1 *Trichoderma viride*	28.88+1.55[b]	46.65+1.84[a]	213.70+13.60[a]
T2 *Trichoderma harzianum*	27.57+0.91[c]	43.52+1.52[b]	200.90+12.61[b]
Trichoderma **species and neem**			
Cake (Interactions) T0N0	35.04± 0.84[a]	22.07±0.34[d]	84.10±1.23[g]
T0N1	32.31±0.38[b]	38.30+0.54[c]	134.30±2.62[f]
T0N2	30.53±0.53[c]	46.57± 0.46[a]	145.10±2.51[ef]
T1N0	22.98±0.99[e]	40.35± 0.47[bc]	163.80±3.15[d]
T1N1	30.66+0.28[c]	49.61± 0.64[a]	220.50± 3.01[c]
T1N2	33.01± 0.47[b]	50.00±3.20[a]	256.80±2.21[a]
T2N0	24.39± 0.42[e]	38.80± 0.20[c]	154.50±3.36[de]
T2N1	27.88± 0.57[d]	42.74±0.5[b]	214.50± 13.07[c]
T2N2	30.43±0.50[c]	49.03± 0.88[a]	233.80± 4.28[b]

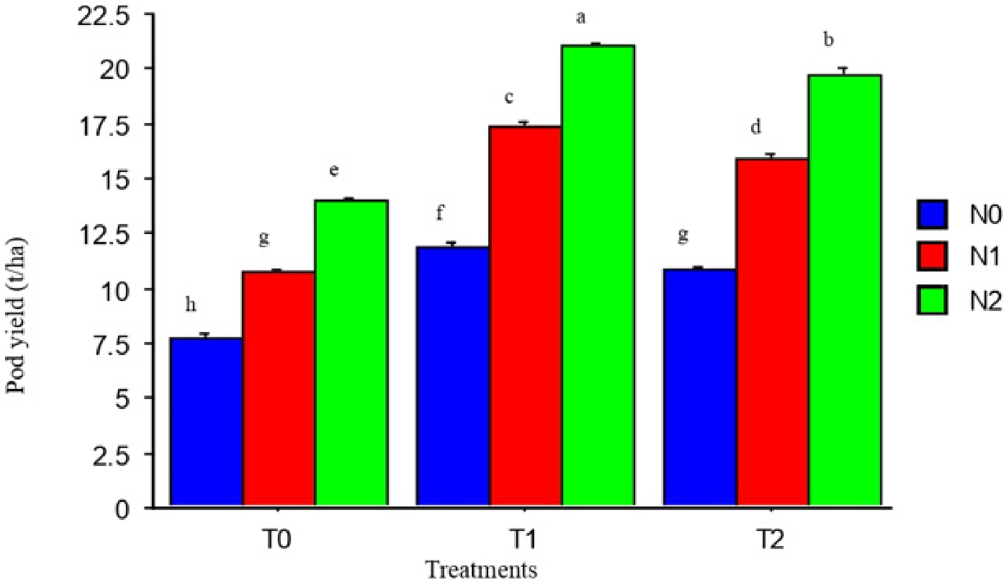

Figure 3: *Interaction effects of Trichoderma species in combination with neem cake on pod yield t/ha a = Significantly (P≥0.05) highest pod yield of common bean plant (ANOVA and significant means were compared using duncan new multiple range test at 5% probability level)*

Table 7: *Effects neem cake (N) Trichoderma species (T) and their interactions on number of pods per plant and pod yield of common bean plant naturally infested with M. incognita and S. rolfsii.*

Neem (t/ha)	Number of pods per plant	Pod yield t/ha
N00.00	8.56±0.50[c]	10.13±0.64[c]
N12.00	11.00±0.55[b]	14.65±1.01[b]
N24.00	12.22±0.52[a]	18.24±1.10[a]
Trichoderma **species (T.)**		
T0 (control)	8.67±0.58[b]	10.78±0.91[c]
T1 *Trichoderma viride*	11.78±0.66[a]	16.77±1.34[a]
T2 *Trichoderma harzianum*	11.33±0.47[a]	15.47±1.29[b]
Trichoderma **species and neem**		
Cake (interactions) T0N0	6.67±0.33[a]	
T0N1	9.00±0.58[a]	
T0N2	10.33±0.33[a]	
T1N0	9.33±0.33[a]	
T1N1	12.33±0.33[a]	
T1N2	13.67±0.33[a]	
T2N0	9.67±0.33[a]	
T2N1	11.67±0.33[a]	
T2N2	12.67±0.33[a]	

highest pod yield. The interaction effects indicated no significant influence (P≥0.05) on the quantity of pods per individual plant. Conversely, the interaction effects on pod yield t/ha revealed a statistically significant enhancement (P≤0.05), with highest pod yield t/ha observed in plants subjected to treatment T1N2.

Discussion

The results of this investigation indicated that the inoculation of *T. viride* and *T. harzianum*, with NC into common bean plants infested with *M. incognita* and *S. rolfsii* exhibited a synergistic effect. Natural field infestations by both pathogens led to pronounced root galling in common bean plants, an increased nematode population per plant, an increased incidence of root-knot disease, and an increase in nematode density within the soil. The significant degree of galling observed in common bean plants may be linked to environmental factors, particularly given that the soil utilized in the field trials had a notable sand content. Previous studies have indicated that nematode activity is amplified in soils characterized by elevated sand proportions (Agu, 2008; Olewe, 2005). The findings of this investigation align with the observations made by Kim *et al*. (2016), that reported that galls are more prevalent on soil composed entirely of sand compared to those with lower sand content.

The incorporation of fungicides and nematicides into soil amendments has been documented as highly effective in controlling soil-borne fungi and nematodes, as well as enhancing soil fertility. Nonetheless, these chemical agents are not environmentally sustainable, prompting the exploration of biocontrol and eco-friendly alternatives as viable strategies for enhancing crop performance via soil amendments (Tanimola, 2008). These organic fertilizers are typically regarded as facilitators of improved crop performance due to several properties inherent to them, such as their capacity to function as nematicides (Sukul, 1992; Kimpinski, 2003), their role in augmenting soil nutrient content, enhancing physical and chemical soil properties, and stimulating the activity of predators and parasites that target phyto-parasitic nematodes (Nico, 2004; Hungalle, 1986). Based on observations from field experiments, the utilization of NC and *Trichoderma* species obviously contributed to reduce galls formation, and the gall index in common beans infected with *M. incognita*. It is conceivable that these fungi produced substances with lethal properties, thereby mitigating the galls induced by *M. incognita*.

Notably, the gall index decreased from an extremely vulnerable level in untreated soil to a resistant level within soils modified utilizing NC, as well as biocontrol agents. Additionally, these populations of nematode per plant, the density of nematode within the soil, as well as reproduction factor of nematode was lowered significantly resulting from the incorporation of neem and *Trichoderma* species into the soil. These results align with previous studies by various authors indicating the addition of neem compost and *Trichoderma* species effectively reduces root galling in addition to population of nematode within both roots as well as soil caused by *M. incognita*, hence, promoting common bean cultivation (Atungwu *et al*., 2009; Chimbekujwo and Neher, 2001; Radwan *et al*., 2012).

Neen cake exhibited greater efficacy at 4.00 t/ha compared to 2.00 t/ha in terms of lowering of galls formation and the number of nematodes in the soil. Akhatar and Mahmood (1995), stated that NC comprises phenolic compounds, tannins, flavonoids, and a variety of chemicals including salanin, thironemonie, and azadirachtin, which possess nematicidal properties at specific concentrations. This finding is in line with earlier reports on the bioactivity of neem leaves and seed extracts against several pathogens (Del Serrone and Nicoletti 2013). In the field trial, it was noted that treatment with NC exerted a substantial suppressive effect on *S. rolfsii* at a dosage of 2.00 t/ha, in contrast to the higher rate of 4.00 t/ha. Given the complexity of the additional nutrients present in the field, it is plausible that treatment with 2.00 t/ha of NC facilitated a more rapid and effective synergy with other soil nutrients, thereby enhancing the control of *S. rolfsii* relative to the higher application rate of 4.00 t/ha.

T. harzianum outperformed *T. viride* in terms of reducing the incidence of *S. rolfsii* disease and the associated disease SI within the field environment. According to the findings reported by Eziashi *et al*. (2007), *T. harzianum* exhibits antagonistic properties against *S. rolfsii* through the production of various bioactive compounds, including viridian, glotoxin, gliotoxin, glioviridin, dermin, and trichodermin. The increased release rate of these bioactive substances by *T. harzianum* into the soil could elucidate the enhanced efficacy observed against *S. rolfsii* compared to *T. viride*. The synergistic effects of NC combined with *Trichoderma* species proved to be more potent in the management of *S. rolfsii* disease incidence and severity during the field experiment. It is conceivable that NC facilitated the release of nematicidal compounds or organic matter that interacted proficiently with the antagonistic attributes of *Trichoderma* species, thereby inhibiting the proliferation of *S. rolfsii*.

In the present investigation, there was a notable improvement in both growth and yield of common bean in soils that were either fortified with NC or inoculated with species of *Trichoderma*, in comparison to the unamended control groups. The crops treated with NC demonstrated greater plant height, with the most notable effect observed at the higher application rate of 4.00 t/ha, measured within 3, 5, as well as 7 weeks then the planting were just concluded. It therefore implies NC enriches soil fertility, which may explain the increase in both plant height and pod yield (t/ha), as nutrients are gradually released into the growing environment. This result supports the observations of Krishnaray *et al*. (2018) and Madhuri *et al*. (2021), who found that NC boosts plant height and pod production in tomato plants. Similarly, Olabiyi *et al*. (2016) reported a significant improvement regarding the plant height of common bean following neem compost application. Consistent with these findings, Eifediyi *et al*. (2013) also noted an increase in common bean plant height following NC treatment.

At the 3-week after planting (WAP), the inoculation of the soil with *Trichoderma* species did not demonstrate any statistically significant effects on plant height in the experimental field. This lack of effect could be linked to the short duration when these observations were made. Comparable results were observed by Olabiyi *et al*. (2016), where the application of *T. harzianum* did not yield a significant effect on the height of common beans relative to the untreated controls. The optimal height of common bean plants was recorded when the soil was treated with *T. viride* in conjunction with NC at the rate of 4.00 t/ha. This finding corroborates the research of Olabiyi *et al*. (2016), who noted that the combination of *T. viride* with organic amendments promoted vigor and growth of common bean plant. The fresh root, dry leaf, and dry stem weights of common beans in this investigation were found to be superior in soils that were enriched with neem and the biocontrol agents, when compared to the unamended plots.

Conclusions and Recommendations

The investigation identified a synergistic relationship between *T. viride* and *T. harzianum,* with NC. The degree of galling and basal stem rot exhibited a marked increase when the two pathogens were simultaneously inoculated compared to their individual inoculation. The study demonstrated that the combination of *T. viride* and 4.00 t/ha of NC was the most effective in managing *M. incognita* and *S. rolfsii* in the cultivation of common bean plants. Treatment significantly lowered gall formation, nematode populations, and disease incidence. *T. viride* outperformed *T. harzianum* in reducing pathogen impact, with treated common bean plants showing enhanced growth, and yielding the highest pod production (21.08 t/ha). The above findings outline integration of biocontrol agents as well as organic amendments for efficient pest management in common bean plant. Upcoming studies have to ascertain long-term treatments effects as well as their usability across various agro-ecological zones.

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Novelty statement

The research has highlighted the potential application of NC and native isolates of *Trichoderma* species cultivated on millet as a strategy for controlling the disease complex affecting common beans, which is induced by *M. incognita* and *S. rolfsii.* Integrating organic amendments and beneficial microorganisms is a promising strategy for managing the disease complex affecting common beans.

Author’s contribution

Daniel Offiong Etim: Designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of manuscript.
Rosemary Anietie Bassey and Glory Akpan Bassey: Managed the analysis of the study.
Ndarake Eden Ini-Ibehe: Managed the literature searches. All authors read and approved the final manuscript.

Generative AI or AI assisted technology statement
The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest
The authors have no conflict of interest.

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