Trop J Nat Prod Res, October 2025; 9(10): 5114 - 5121

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Evaluating Pre-planting *Trichoderma asperellum* Application for Biocontrol of *Macrophomina phaseolina* in Screenhouse-Grown Cowpea

Olusola L. Oyesola [1, 2], Tonjock R. Kinge [4], Olufisayo A. Kolade [5], Olawole O. Obembe [1, 2, 3]*

[1]*Department of Biological Sciences, College of Science and Technology, Covenant University, P.M.B. 1023, Ota, Ogun State, Nigeria*
[2]*Plant Science Research Cluster, Covenant University, Ota, Nigeria*
[3]*UNESCO Chair on Plant Biotechnology, Covenant University, Nigeria*
[4]*Department of Plant Sciences, Faculty of Science, the University of Bamenda, P.O.Box 39, Bamenda, Cameroon*
[5]*Virology Unit, IITA, PMB 5320, Moniya, Oyo Road, Ibadan*

ARTICLE INFO

Article history:
Received 18 April 2025
Revised 13 September 2025
Accepted 14 September 2025
Published online 01 November 2025

ABSTRACT

Cowpea is recognised for its importance as a food and forage crop for animals. However, *Macrophomina phaseolina*, a fungus that causes pre-harvest crop loss, affects its production. In this study, *Trichoderma* was employed as an alternative to synthetic fungicides that negatively impact biodiversity to manage rot disease in cowpea. Three strains *of Trichoderma asperellum* were isolated from the soil. The spore suspensions of the *Trichoderma* strains were formulated into seven treatment combinations and applied to the cowpea soil before planting to investigate their biocontrol potential on *M. phaseolina* and their effects on cowpea biomass. The result showed that Trt3 (54.5417 cm), Trt1 (54.0625 cm) and Trt4 (52.8250 cm) had higher plant height than the negative control (*M. phaseolina* only (44.9667 cm)). Also, Trt7 (0.5446 cm) and Trt5 (0.5313 cm) had a higher stem girth performance than in the negative control (*M. phaseolina* only (0.3333 cm)), while Trt7 (24.958), Trt3 (21.417) and Trt6 (20.083) recorded a higher leaf number than in the negative control (*M. phaseolina* only (8.833)). Zero disease incidence was observed in Trt3 (0%) and Trt7 (0%) upon treatment with the *Trichoderma* formulations. Zero disease severity was recorded in Trt3 and Trt7 (0%), compared to the negative controls, which displayed 100% disease incidence and severity. The pre-planting *Trichoderma* application enhanced cowpea biomass and reduced disease incidence and severity compared to the negative control. Therefore, *Trichoderma* is an effective bioagent for controlling diseases caused by *M. phaseolina* in cowpea and stimulating its overall performance.

Keywords: *Trichoderma asperellum*, *Macrophomina phaseolina*, Pre-planting, Biocontrol, Bioformulation, Plant diseases

Introduction

Economic crops provide essential resources that solve global food insecurity through horticulture and agricultural platforms. [1, 2, 3] Cowpea is one of the significant economic crops grown mainly in Nigeria and many other West African countries to combat the menace of food insecurity. [4, 5] However, the significant production of cowpeas is affected by several biotic factors, which include diseases. [6, 7] The emergence and proliferation of plant diseases, predominantly observed in the fields of horticulture and forestry, have profound global consequences for agricultural producers, the seed sector, policymakers, and the broader populace, [8] as well as for global food security and the loss of biodiversity in numerous vulnerable regions worldwide. [9] These disease incidences lead to reductions in crop yields and ecological damage. For instance, the annual reduction in crop yields attributed to pathogens (microorganisms responsible for causing diseases and affecting the health and productivity of hosts) and pests alone is quantified at US$220 billion, directly affecting food security, regional economies, and various interconnected socio-economic dimensions. [10]

Corresponding author: Email: olawole.obembe@covenantuniversity.edu.ng Tel: +234(0)8130928965

Citation Oyesola L O, Kinge T R, Kolade A O, Obembe O O. Evaluating Pre-planting *Trichoderma* application for Biocontrol of *Macrophomina phaseolina* in Screenhouse-Grown Cowpea. Trop J Nat Prod Res. 2025; 9(10): 5114 – 5121 https://doi.org/10.26538/tjnpr/v9i10.55

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Nigeria has fertile agricultural land spanning 34 million hectares, with 70% of its workforce engaged in the agricultural sector. [11] Despite this abundance, the country has consistently grappled with the issue of insufficient food production. Annually, over 40% of crop yield is lost due to pest infestations and diseases, [12, 13,] rendering a significant portion of the planted food crops inedible before they reach the market. [14] This situation forces farmers to resort to chemical pesticides despite their harmful effects on non-target organisms, including beneficial insects, livestock, and humans. [15] Consequently, both the quality of food produced and the economic stability of numerous resource-poor farmers—who play a critical role in supplying a substantial proportion of local food—are increasingly undermined. [16] Achieving sustainable economic development in Nigeria remains an elusive goal without a focus on ensuring the well-being and health of its population. [17] With a projected population growth rate of 2.5% and an anticipated population of 400 million by 2050, it is imperative to prioritise initiatives that enhance both the quality and quantity of food production in Nigeria. This is essential to avert a looming food insecurity crisis that could result in a significant decline in the population. [17, 18]

Macrophomina phaseolina is a prevalent soil-borne fungal pathogen distributed worldwide, with the ability to affect over 500 plant species across more than 100 taxonomic families. Its broad host range and widespread occurrence underscore the significant agricultural threat it poses. This root-infecting fungus causes blockages in root vascular tissues, leading to plant death.[19] This fungus is known to cause several severe diseases, including stem and root rot, charcoal rot, and seedling

blight. [20] This pathogen can severely affect crop yields under elevated temperatures ranging from 30 to 35 °C, combined with low soil moisture levels (below 60%). This is especially true for critical agricultural staples such as cowpea, soybean, groundnut, and sorghum. The resulting crop losses can significantly undermine farmers' incomes, seriously threatening their livelihoods and overall food security in the affected regions. Addressing these environmental factors is crucial for maintaining healthy crops and protecting agricultural productivity. [21] In the most severe cases, complete crop failure has been observed when the disease manifests during the pre-emergence stage. [22] It can persist in the soil for extended periods, utilising microsclerotia or chlamydospores as its survival mechanisms. This longevity allows it to cause infections, primarily due to the subsequent development of sclerotia, which are thick, hardened structures formed in response to environmental stresses. [19] Despite numerous research efforts to control this pathogen, developing effective management strategies remains challenging due to the fungus' ability to remain dormant in farm soil for extended periods. [20]

Despite the use of various techniques, including soil fumigation, solarisation, resistant cultivars, and biological, cultural, physical, and chemical control, managing soil-borne diseases can sometimes be difficult. [19, 23] Farmers have been using synthetic pesticides to control plant diseases, which, while effective, harm biological diversity because they can alter habitats and the food chain, which can have long-term and toxic short-term effects on organisms directly exposed. [24] After decades of widespread application in agriculture, researchers uncovered the alarming toxicity of certain pesticides and their tendency to accumulate in soils, water sources, and the food chain. This accumulation poses a serious threat not only to human health but also to wildlife. [25] The detrimental effects of these chemicals have been a significant factor in the ongoing depletion of biodiversity, leading to the extinction of various species, the loss of natural resources, and the destruction of habitats. As ecosystems become destabilised, the balance necessary for sustaining life becomes increasingly compromised, highlighting the urgent need for sustainable farming practices. [26]

Research has recently focused on creating environmentally friendly biocontrol strategies for efficiently managing plant disease to lessen the detrimental effects of synthetic pesticides on biodiversity. [27] *Trichoderma* is one of the potent biocontrol agents that has shown promise in managing pathogens that cause plant diseases. [28] *Trichoderma* is frequently used as a biocontrol agent to protect crops from soil-borne pathogens. [29] *Trichoderma* directly controls pathogens through mechanisms like mycoparasitism or indirectly through competing with other plants for nutrients and space, altering the environment, or encouraging plant growth and defence mechanisms, increasing the uptake of nutrients and the general vigour of the plant. [30, 31, 32.] *Trichoderma* is known for its effectiveness and versatility, rendering it an invaluable asset in integrated pest management and sustainable agricultural practices. This genus of beneficial fungi plays a significant role in enhancing plant health through the biocontrol of various pathogens and the improvement of soil quality. Multiple species of *Trichoderma*, such as *T. viride*, *T. koningii*, *T. harzianum*, *T. longibrachiatum*, and *T. virens*, have been thoroughly researched for their effectiveness in biocontrol of plant diseases. These species not only inhibit harmful fungal pathogens but also enhance plant resistance to diseases, promote root development, and improve nutrient absorption. Consequently, they play a vital role in fostering more sustainable and efficient crop production systems. [33, 34] Therefore, it was essential to carry out this research to determine the effectiveness of the pre-planting soil application of single and combined *Trichoderma* spores' suspension on managing *Macrophomina phaseolina* and improving cowpea biomass.

Materials and Methods

Cowpea seed collection

Viable cowpea seeds (accession number IT13K-1308-5) were collected from the Germplasm Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria, on the 30th of November, 2023.

Macrophomina phaseolina collection

The pure stock culture of *Macrophomia phaseolina* was obtained from the International Institute of Tropical Agriculture (IITA) Pathology Laboratory, Ibadan, Oyo State, Nigeria. The culture was refreshed by growing on PDA media and allowed to grow in the incubator at 27 ^{0}C for 7 days before use for the experiment. [35]

Isolation and morphological characterisation of Trichoderma isolates

Soil sample collection and suspension preparation

Soil samples were randomly collected from Covenant University Parks and Gardens, Ota, Ogun State. The samples were carefully transported to the Biology Laboratory within the Department of Biological Sciences at Covenant University, utilising sterile Ziploc bags to ensure their integrity. The soil samples were homogenised, and 1 g was weighed using an analytical weight balance to isolate *Trichoderma* using the serial dilution method (10^{-3}, 10^{-5}, 10^{-7}). [36]

Media preparation

Potato Dextrose Agar (PDA) media, sourced from DIFCO, was prepared according to the guidelines of the manufacturer, ensuring the optimal conditions for cultivating fungi and yeast. A total of 39 g of PDA was measured and dissolved in a conical flask containing 1000 mL of distilled water. The conical flasks were plugged with cotton wool coated with aluminium foil and sealed with masking tape. The media was homogenised by agitating on a rotatory shaker for 10 min at 200 rpm. The media was sterilised for 15 min at 121 ℃ using an autoclave. The PDA was allowed to cool down to about 40 ℃, after which 2 - 3 drops of Lactic acid were added to effectively lower the pH of the media, creating an unfavourable condition for the proliferation of pathogenic bacteria. [30].

Isolation of Trichoderma

A 2.5 mL aliquot from the soil serial dilution preparation was dispensed into a prepared PDA media plate and incubated for seven days at 27 ºC, using a Genlab classic incubator (United Kingdom). The pure cultures of *Trichoderma* isolates were obtained from the mixed cultures by subculturing distinct colonies in freshly prepared media. Pure isolates were further obtained via distinct colony selection on PDA. The isolates were refreshed by subculturing in media before initiating the experiment. [35]

Characterisation of fungal isolates

The fungal isolates were identified using standardised methods for phenotypic and genotypic characteristics. [37] The macro properties of the fungal isolates comprised growth traits, including wooliness and fluffiness, as well as the creation of concentric rings (1–2) and colours ranging from white to yellowish green to green and dark green during conidia production. The fungal isolates were genotypically characterised by amplifying the Internal Transcribed Spacer (ITS) region using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'TCCTCCGCTTA TTGATATGC-3') primers. The fungal isolates were identified as *Trichoderma asperellum* Tric4 (accession number - PV491559), *Trichoderma asperellum* Tric12 (accession number - PV491560), *Trichoderma asperellum* Tric13 (accession number - PV491561). The fungal pathogen was identified as *Macrophomina phaseolina* (accession number - PV491558).

Spore suspension preparation

The spores from a 7-day-old culture of *Trichoderma* and *Macrophomina phaseolina* isolates were carefully transferred into a 700 mL conical flask containing sterile distilled water. This was accomplished by rinsing the cultures with an additional 50 mL of sterile distilled water, ensuring the effective collection of spores. Following this procedure, the spore suspensions of the *Trichoderma* isolates, along with the *Macrophomina phaseolina* spores, were stored in a refrigerator at a temperature of 4 ºC. This process was conducted according to the modified methods outlined by Oyewole *et al*., [38] ensuring the integrity and viability of the fungal cultures for further experimentation.

Treatment combinations with controls

The *Trichoderma* suspensions prepared from *Trichoderma asperellum* Tric4, *Trichoderma asperellum* Tric12, and *Trichoderma asperellum*

Tric13 were combined by mixing 50 mL from each *Trichoderma* suspension using the formulation method in Table 1 according to the revised procedure of Chen *et al*. [39,] to derive the treatments (Trt1, Trt2, Trt3, Trt4, Trt5, Trt6, and Trt7) applied in the screenhouse. Seven treatments were adopted for the experiment, with two control experiments (positive and negative controls) as presented in Table 2. Each treatment was set up in triplicate.

Soil collection and sterilisation

The topsoil used for the screenhouse experiment was collected in January 2024 from the Covenant University parks and garden from 0-20 cm depth using a shovel. It was conveyed to the Biology Laboratory of the Department of Biological Sciences in aluminium containers. The soil was then sterilised by exposure to high temperature using an autoclave (Vertical Pressure Steam Steriliser, Model: LS-50LJ) at 121 ºC for 90 min. After sterilising, the soil was left to cool before handling and loosened to restore its natural structure. A 2 kg sterilised soil was transferred into the planting pots, and the pots were tapped to settle the soil and remove air pockets. The planting pots were arranged, ensuring adequate spacing for maximum air circulation.

Cowpea seed planting

Before planting, the planting pots were thoroughly moistened to ensure optimal seed germination. Seed planting was done on the 2nd of February 2024. In each pot, three cowpea seeds were carefully placed at a depth of approximately 1 to 2 centimetres. After planting, the seeds were covered with a thin layer of soil to protect them while allowing for adequate airflow and moisture retention. Once germination had occurred, the seedlings were carefully thinned to one seedling per pot by removing the physically weaker seedlings. A clean environment was maintained to prevent infestation and disease transmission.

Soil inoculation with Trichoderma and Macrophomina phaseolina

Cowpea soil in the screenhouse was inoculated with 50 mL spore suspension of *Trichoderma* and *M. phaseolina* isolates according to the modified procedure of Afouda *et al*. [40] The *Trichoderma* and *M. phaseolina* suspension solution was poured over the soil surface to ensure uniform coverage. The soil was gently mixed to a 5-10 cm depth to ensure even distribution of the *Trichoderma*.

Measurement of Disease incidence and severity

The percentage of disease incidence observed in the infected cowpea seedlings was assessed across the treatment groups, following the methodology outlined by Persson *et al*. [41]

$$\text{Disease incidence (\%)} = \frac{\text{number of plants showing disease symptoms}}{\text{Total Number of seedlings}} \times 100 \quad \text{(equation 1)}$$

The percentage of disease severity in the infected cowpea seedlings across the treatments was determined using the modified scale of Persson *et al*. [41] as presented in Table 3. The data was collected once from each treatment representative, in the last week of assessment, before the termination of the experiment.

Experimental design

The experiment was a Completely Randomised Design of 7 × 1 × 3 (i.e., 7 treatments × 1 application type × 3 replicates) with 2 controls (soil treated with *Macrophomina phaseolina* alone [negative control] and cowpea planted alone [positive control]) also in triplicate. The design had twenty-one (21) experimental units and six (6) control units.

Data collection and statistical analysis

The biomass data were collected at 5-day intervals by observing plant height (cm), leaf number, and stem girth (cm). The collected data were subjected to variance analysis using the Statistical Analysis System (SAS 9.4) software package. The means were compared using the Least Significant Difference (LSD) at a 0.05 confidence interval. This design allowed for the evaluation of the effects of the *Trichoderma* isolates on *Macrophomina phaseolina*, with the control serving as a baseline for comparison.

Results and Discussion

Effect of treatments on cowpea performance under pre-planting application type

The introduction and growing utilisation of biological control agents in managing plant diseases have emerged as a sustainable and eco-friendly alternative to conventional synthetic pesticides. Unlike their chemical counterparts, which can adversely affect ecosystems and diminish biodiversity, these natural interventions harness the potential of beneficial organisms to promote plant health while safeguarding the environment. This study revealed the effectiveness of the pre-planting application of combined *Trichoderma* spores' suspension against *Macrophomia phaseolina* in the screenhouse. Table 4 shows the biomass performance of the cowpea subjected to the pre-planting *Trichoderma* application. It shows a significant difference in the model, treatment, and growth progress ($p<.0001$). However, replicates had no significant difference ($p>0.9470$). The means comparison of cowpea

Table 1: *Trichoderma* spores suspension formulation

Treatments	Formulations
Trt1	*T. asperellum* Tric4
Trt2	*T. asperellum* Tric12
Trt3	*T. asperellum* Tric13
Trt4	*T. asperellum* Tric4 + *T. asperellum* Tric12
Trt5	*T. asperellum* Tric4 +*T. asperellum* Tric13
Trt6	*T. asperellum* Tric12 + *T. asperellum* Tric13
Trt7	*T. asperellum* Tric4 + *T. asperellum* Tric12 + *T. asperellum* Tric13

Table 2: *Trichoderma* treatments application with *M. phaseolina* and control

Treatment	Formulations
Trt1	Cowpea + *M. phaseolina* + *T. asperellum* Tric4
Trt2	Cowpea + *M. phaseolina* + *T. asperellum* Tric12
Trt3	Cowpea + *M. phaseolina* + *T. asperellum* Tric13
Trt4	Cowpea + *M. phaseolina* + *T. asperellum* Tric4 + *T. asperellum* Tric12
Trt5	Cowpea + *M. phaseolina* + *T. asperellum* Tric4 + *T. asperellum* Tric13
Trt6	Cowpea + *M. phaseolina* + *T. asperellum* Tric12 + *T. asperellum* Tric13
Trt7	Cowpea + *M. phaseolina* + *T. asperellum* Tric4 + *T. asperellum* Tric12 + *T.asperellum* Tric13
Trt8	Control 1 – Cowpea alone
Trt9	Control 2 - Cowpea + *M. phaseolina*

Table 3: Disease rating scale

Percentage value	Plant area infected
0	Symptomless plant
5	5 mm lower stem discolouration or less

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

10	20 mm lower stem discolouration
25	5% lower stem discolouration
50	Whole stem discolouration, but symptomless epicotyls or leaves
75	Whole stem and epicotyls discolouration with the lower leaves wilted
100	Dead plants

plant height (cm), stem girth (cm) and leaf numbers under the treatments were shown in Tables 5, 6 and 7, respectively. Table 5 shows a significant difference in cowpea heights under the different treatments. Trt3 (*T. asperellum* Tric13) gave the highest cowpea height mean (54.5417 cm), followed by Trt1 (*T. asperellum* Tric4) with 54.0625 cm and Trt4 (*T. asperellum* Tric4 + *T. asperellum* Tric12) with 52.8250 cm, which did not differ significantly in their performance. However, all the treatments gave better cowpea plant height than those treated with *M. phaseolina* (44.9667 cm). Also, Table 6 shows a significant difference in cowpea stem girths under the different treatments. Trt7 (*T. asperellum* Tric4 + *T. asperellum* Tric12 + *T. asperellum* Tric13) and Trt5 (*T. asperellum* Tric4 + *T. asperellum*

Table 4: ANOVA for the effects of treatments on cowpea biomass under the pre-planting application type

Plant height (cm)							
Source		DF	SS	MS	F Value	Pr > F	
Model		10	38767.71	2280.45	243.50	<.0001	
Treatment	8	1553.34	194.17	20.73	<.0001		
Replicate	2	1.02	0.51	0.05	0.9470		
Error		198	1854.36	9.37			
Corrected Total	208	40622.07					
Stem girth (cm)							
Model		10	7.13	0.42	113.90		<.0001
Treatment	8	0.85	0.11	28.87	<.0001		
Replicate	2	0.00	0.00	0.27	0.7650		
Error		198	0.73	0.00			
Corrected Total	208	7.86					
Leaf number							
Model		10	17920.21	1054.13	11.82	<.0001	
Treatment	8	5512.87	689.11	7.73	<.0001		
Replicate	2	483.12	241.56	2.71	0.0691		
Error		198	17656.82	89.18			
Corrected Total	208	35577.04					
(<0.05)							

Table 5: Mean comparison for the effects of treatments on cowpea plant height (cm) under the pre-planting application type

Grouping	**Mean**	**Treatment**
A	54.5417	Trt3
AB	54.0625	Trt1
AB	52.8250	Trt4
B		
BC	52.4000	Trt7
C		
CD	50.8917	Trt6
CD	50.6625	Trt5
D	50.5708	Trt9
D	49.9167	Trt2

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

E	44.9667	Trt8
R^2	0.1243	
$LSD_{0.05}$	1.7421	

Table 6: Mean comparison for the effects of treatments on cowpea stem girth (cm) under the pre-planting application type

Grouping	Mean	Treatment
A	0.5446	Trt7
A	0.5313	Trt5
AB	0.5167	Trt1
AB	0.5138	Trt6
B	0.4917	Trt2
B	0.4875	Trt3
B	0.4792	Trt4
C	0.4133	Trt9
D	0.3333	Trt8
R^2	0.1243	
$LSD_{0.05}$	0.0345	

Table 7: Mean comparison for the effects of treatments on cowpea leaf number under the pre-planting application type

Grouping	Mean	Treatment
A	25.958	Trt9
AB	24.958	Trt7
B		
BC	21.417	Trt3
BC	20.083	Trt6
C		
CD	19.042	Trt2
CD	18.083	Trt4
CD	16.208	Trt5
D		
DE	13.750	Trt1
E	8.833	Trt8
R^2	0.124	
$LSD_{0.05}$	5.376	

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

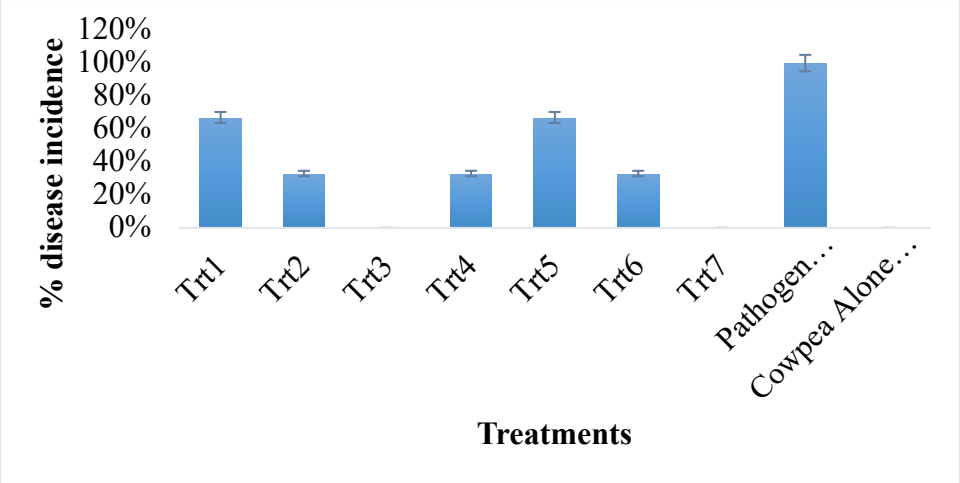

Figure 1: Effect of *Trichoderma* treatments on cowpea disease incidence

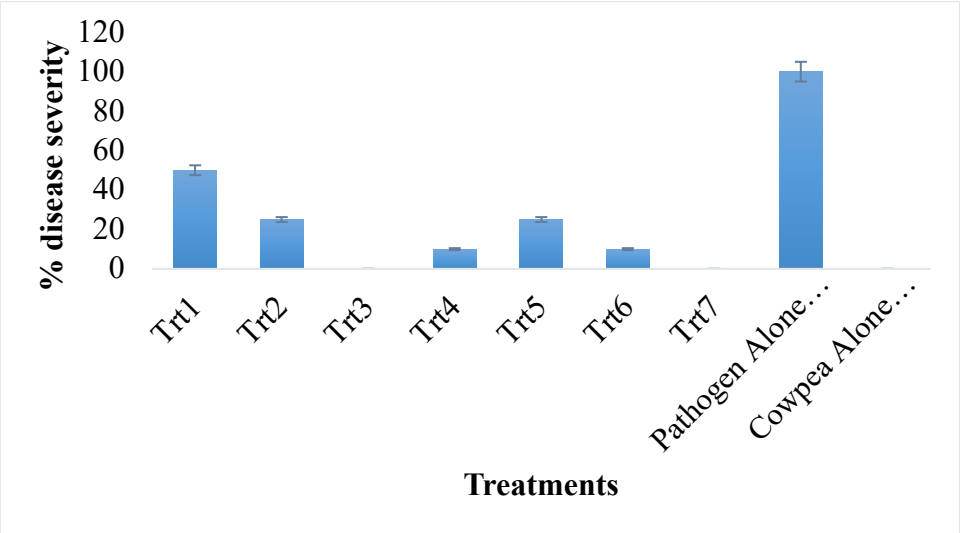

Figure 2: Effect of *Trichoderma* treatments on cowpea disease severity

Tric13) gave the highest cowpea height mean, 0.5446 cm and 0.5313 cm, respectively, followed by Trt1 (*T. asperellum* Tric4) and Trt6 (*T. asperellum* Tric12 + *T. asperellum* Tric13), with 0.5167 cm and 0.5138 cm, respectively, which did not differ significantly in their performance. However, all the treatments gave better cowpea stem girth than those treated with *M. phaseolina* (44.9667). Table 7 shows a significant difference in cowpea leaf number under the different treatments. Trt9 (control 2 - cowpea alone) (25.958) and Trt7 (24.958) gave the highest leaf number, followed by Trt3 (21.417) and Trt6 (20.083), which did not differ significantly in their performance. However, all the treatments gave better cowpea leaf numbers than those treated with *M. phaseolina* (8.833). The study further showed that *Trichoderma* has significant antifungal potential against *M. phaseolina*, which causes charcoal root/stem rot in cowpeas. Poveda [42] revealed that *Trichoderma* species can effectively manage their target fungal species through a variety of specific mechanisms. These mechanisms include the production of enzymes that degrade fungal cell walls, the release of secondary metabolites that inhibit fungal growth, and the establishment of competitive interactions that limit the resources available to pathogenic fungi, which cluster around the plant rhizosphere. Such multifaceted approaches highlight the ecological significance of *Trichoderma* in sustainable agriculture and plant health management. The spore suspension culture of *T. asperellum* Tric4, *T. asperellum* Tric12*, and T. asperellum* Tric13 tested showed high antifungal activity against *M. phaseolina* artificially inoculated into the cowpea planting soil.

The synergistic ability of the different *Trichoderma* strains was proven in the performance of the cowpea, where the combination of *T. asperellum* Tric4, *T. asperellum* Tric12*, and T. asperellum* Tric13 gave higher cowpea biomass in the cowpea stem girth and leaf number than in the cowpea treated with a single strain of *Trichoderma*. The lower performance of the cowpea plant height observed in cowpea treated with combined *Trichoderma* strains in comparison with the single *Trichoderma* application might have resulted from increased *Trichoderma* PGPS (plant growth promoting substances) due to the combination of multiple *Trichoderma* strains, which, according to the findings of Nieto-Jacobo *et al*. [43] and Guzman-Guzman *et al*., [44], reduces internode length, leading to shorter plants. This result agrees with the findings of the report of Napolitano *et al*., [45] which showed the effectiveness of *Trichoderma*-based bioformulation from two different *Trichoderma* strains in managing *Fusarium oxysporum, Sclerotinia sclerotiorum*, *Botrytis cinerea*, and *Aspergillus* sp.

Measurement of Disease incidence and severity

The effects of pre-planting *Trichoderma asperellum* bioformulation on the incidence and severity of *Macrophomina phaseolina* fungal disease in cowpea were investigated and recorded. Figure 1 shows the ability of *Trichoderma* to reduce the incidence of disease caused by *M. phaseolina* in the cowpea plants treated with different *Trichoderma* formulations. The disease incidence assessment shows that all the

treated cowpeas had a lower incidence than the untreated cowpea (pathogen alone – control 1), which had a 100% incidence. The cowpea treated with Trt3 and Trt7 had a 0% disease incidence, and the cowpea treated with Trt2, Trt4, and Trt6 had a 33% incidence. Meanwhile, the cowpea treated with Trt1 and Trt5 had a 67% disease incidence. Figure 2 shows *Trichoderma*'s potential to lessen the severity of disease triggered by *M. phaseolina* in the cowpea plants treated with different *Trichoderma* formulations. The disease severity assessment shows that all the treated cowpeas had a lower severity than the untreated cowpea (pathogen alone – control 1), which had a 100% severity. The cowpea treated with Trt3 and Trt7 had a 0% disease severity, and the cowpea treated with Trt4 and Trt6 had a 10% severity, and Trt5 had a 25% severity. Meanwhile, the cowpea treated with Trt1 had a 50% disease severity.

The single application of the *Trichoderma* strains proved effective in controlling *M. phaseolina.* This suggests that single or combined *Trichoderma* forms could effectively manage the disease. The untreated control cowpea showed significantly high disease severity, ranging from chlorosis, wilting, and dark colouration in the root and lower shoots in the early disease stage and total cowpea plant death at the latter stage during the growth and development stage, thus causing loss of biomass in the cowpea plants. These disease symptoms may be implicated in substantial pre-harvest cowpea loss, affecting their yield volume and marketability due to low quality. A similar result was documented in the report of Jabeen *et al*. [19] However, the treated cowpea plants exhibited lower disease incidence and severity than the untreated control 1 plants. From this study, the pre-planting application method of *Trichoderma* to planting soil inhibited *M. phaseolina* disease development in the soil; this is supported by the reports of Kumari *et al*. [46] and Waleed *et al*. [47] Furthermore, the pre-planting application of the *Trichoderma* formulations to the cowpea might have increased its biomass performance, as noted in the cowpea plant height and stem girth, when compared with the biomass performance of the cowpea (cowpea alone – control 2) that did not receive *Trichoderma* treatment. This was corroborated by the report of Zin and Badaluddin [48] who reported the ability of *Trichoderma* to enhance the growth of plants. Generally, pre-planting soil application of the single and combined *Trichoderma* formulation proved effective in managing charcoal rot disease caused by *M. phaseolina* and improving cowpea growth.

Conclusion

This investigation indicated that *Trichoderma asperellum* strains could be an effective bio-management alternative to managing charcoal rot in cowpea plants. The study also highlighted the improved effectiveness of combined applications of *Trichoderma* in plant disease management. Besides suppressing *M. phaseolina*, *Trichoderma asperellum* acted as a growth promoter and significantly improved the growth and biomass of cowpea plants grown in the infested soil. However, exploring the mechanisms of action of *Trichoderma* in the rhizosphere is essential for understanding its biocontrol mode in the soil. Furthermore, future research should focus on improving the formulation as a ready-to-use product by farmers on the farm for controlling soilborne fungi pathogens.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors would like to express their sincere gratitude to the Covenant University Centre for Research, Innovation, and Discovery (CUCRID) for funding the publication of this project with grant number CSG/0116/24/01. Special thanks are also extended to the International Institute of Tropical Agriculture (IITA), Oyo State, which provided the *Macrophomina phaseolina* stock culture for this research.

References

1. Obembe OO, Popoola JO, Leelavathi S, Reddy SV. Advances in plant molecular farming. Biotechnol Adv. 2011; 29(2):210-22. doi:10.1016/j.biotechadv.2010.11.004
2. Popoola JO, Obembe OO. Local knowledge, use pattern and geographical distribution of *Moringa oleifera* Lam. (Moringaceae) in Nigeria. J Ethnopharmacol. 2013; 150(2):682-91. doi:10.1016/j.jep.2013.09.043
3. Oluwole OO, Aworunse OS, Aina AI, Oyesola OL, Popoola JO, Oyatomi OA, Abberton MT, Obembe OO. A review of biotechnological approaches towards crop improvement in African yam bean (*Sphenostylis stenocarpa* Hochst. Ex A. Rich.). Heliyon. 2021; 7(11):1-9. e08481. doi:10.1016/j.heliyon.2021.e08481
4. Anago FN, Agbangba EC, Oussou BT, Dagbenonbakin GD, Amadji LG. Cultivation of Cowpea Challenges in West Africa for Food Security: Analysis of Factors Driving Yield Gap in Benin. Agronomy. 2021; 11(6):1139. doi:10.3390/agronomy11061139
5. Baoua I, Rabé MM, Murdock LL, Baributsa D. Cowpea production constraints on smallholders' farms in Maradi and Zinder regions, Niger. Crop Prot. 2021; 142:105533. doi:10.1016/j.cropro.2021.105533
6. Nkomo GV, Sedibe MM, Mofokeng MA. Production Constraints and Improvement Strategies of Cowpea (*Vigna unguiculata* L. Walp.) Genotypes for Drought Tolerance.[4] Int J Agron. 2021; 2021(1):5536417. doi:10.1155/2021/5536417
7. Mohammed SB, Dzidzienyo DK, Umar ML, Ishiyaku MF, Tongoona PB, Gracen V. Appraisal of cowpea cropping systems and farmers' perceptions of production constraints and preferences in the dry savannah areas of Nigeria. CABI Agric Biosci. 2021; 2:25. doi:10.1186/s43170-021-00046-7
8. Ristaino JB, Anderson PK, Bebber DP, Brauman KA, Cunniffe NJ, Fedoroff NV, Finegold C, Garrett KA, Gilligan CA, Jones CM, Martin MD, MacDonald GK, Neenan P, Records A, Schmale DG, Tateosian L, Wei Q. The persistent threat of emerging plant disease pandemics to global food security. Proc Natl Acad Sci U S A. 2021; 118(23). doi:10.1073/pnas.2022239118
9. Mwangi RW, Mustafa M, Charles K, Wagara IW, Kappel N. Selected emerging and reemerging plant pathogens affecting the food basket: A threat to food security. J Agric Food Res. 2023; 14:100827. doi:10.1016/j.jafr.2023.100827
10. Singh BK, Egidi E, Guirado E, Leach JE, Liu H, Trivedi P. Climate change impacts on plant pathogens, food security and paths forward. Nat Rev Microbiol. 2023; 21(10):640-56. doi:10.1038/s41579-023-00900-7
11. Agriculture in Nigeria [Internet]. Wikimedia Foundation. [cited 2024 Apr 15]. Available from: https://en.wikipedia.org/wiki/Agriculture_in_Nigeria
12. Zhang W, Kato E, Bianchi F, Bhandary P, Gort G, Van der Werf W. Farmers' perceptions of crop pest severity in Nigeria are associated with landscape, agronomic and socio-economic factors. Agric Ecosyst Environ. 2018; 259:159-67. doi:10.1016/j.agee.2018.03.004
13. Ofuya TI, Okunlola AI, Mbata GN. A Review of Insect Pest Management in Vegetable Crop Production in Nigeria. Insects. 2023; 14(2):111. doi:10.3390/insects14020111
14. Benjamin J, Idowu O, Babalola OK, Oziegbe EV, Oyedokun DO, Akinyemi AM, Adebayo A. Cereal production in Africa: the threat of certain pests and weeds in a changing climate—a review. Agric & Food Secur. 2024; 13(18):1-16. doi:10.1186/s40066-024-00470-8
15. Tudi M, Daniel RH, Wang L, Lyu J, Sadler R, Connell D, Chu C, Phung DT. Agriculture Development, Pesticide Application and Its Impact on the Environment. Int J

Environ Res Public Health. 2021; 18(3):1112. doi:10.3390/ijerph18031112
16. Andersson E, Isgren E. Gambling in the garden: Pesticide use and risk exposure in Ugandan smallholder farming. J Rural Stud. 2021; 82:76-86. doi:10.1016/j.jrurstud.2021.01.013
17. Abubakar I, Dalglish SL, Angell B, Sanuade O, Abimbola S, Adamu AL, Adetifa IMO, Colbourn T, Ogunlesi AO, Onwujekwe O, Owoaje ET, Okeke IN, Adeyemo A, Aliyu G, Aliyu MH, Aliyu SH, Ameh EA, Archibong B, Ezeh A, Gadanya MA, Ihekweazu C, Ihekweazu V, Iliyasu Z, Kwaku Chiroma A, Mabayoje DA, Nasir Sambo M, Obaro S, Yinka-Ogunleye A, Okonofua F, Oni T, Onyimadu O, Pate MA, Salako BL, Shuaib F, Tsiga-Ahmed F, Zanna FH. The Lancet Nigeria Commission: Investing in health and the future of the nation. Lancet (London, England). 2022; 399(10330):1155-200. doi:10.1016/S0140-6736(21)02488-0
18. Adeyemo JT, Ahmed A, Abaver DT, Riyadh HA, Tabash MI, Lawal AI. Technological Innovation and Agricultural Productivity in Nigeria Amidst Oil Transition: ARDL Analysis. *Economies*. 2024; 12(9):253. https://doi.org/10.3390/economies12090253
19. Shaista J, Marium T, Rubina A, Asma H, Khurram S, Muha mmad WUR, Shahnaz D, Daniel Agricultural Production: Prospects, Applications and Challenges. Molecules. 2021; 26(16):4835. doi:10.3390/molecules26164835
25. Alengebawy A, Abdelkhalek ST, Qureshi SR, Wang Q. Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. Toxics. 2021; 9(3):42. doi:10.3390/toxics9030042
26. Kaur R, Choudhary D, Bali S, Bandral SS, Singh V, Ahmad MA, Rani N, Singh TG, Chandrasekaran B. Pesticides: An alarming detrimental to health and environment. Sci Total Environ. 2024; 915:170113. doi:10.1016/j.scitotenv.2024.170113
27. Kinge TR, Besong PN. Fungi Diversity on Some Fruits and Biological Control using Two Plants Extracts. J Adv Biol Biotechnol. 2021; 24(4):24-38.
28. Olowe OM, Nicola L, Asemoloye MD, Akanmu AO, Babalola OO. *Trichoderma*: Potential bio-resource for the management of tomato root rot diseases in Africa. Microbiol Res. 2022; 257:126978. doi:10.1016/j.micres.2022.126978
29. Yao X, Guo H, Zhang K, Zhao M, Ruan J, Chen J. *Trichoderma* and its role in biological control of plant fungal and nematode disease. Front Microbiol. 2023; 14:1160551. doi:10.3389/fmicb.2023.1160551
30. Oyesola OL, Sobowale AA, Obembe OO. Effectiveness of *Trichoderma koningii* Extract on *Aspergillus* Species Isolated from Rotting Tomato (*Solanum lycopersicum* Mill). Trop J Nat Prod Res. 2020; 4(11):961-965. doi.org/10.26538/tjnpr/v4i11.19
31. Abdullah NS, Doni F, Mispan MS, Saiman MZ, Yusuf YM, Oke MA, Suhaimi NSM. Harnessing *Trichoderma* in Agriculture for Productivity and Sustainability. Agronomy. 2021; 11(12):2559. doi:10.3390/agronomy11122559
32. Ma Y, Li Y, Yang S, Li Y, Zhu Z. Biocontrol Potential of *Trichoderma asperellum* Strain 576 against *Exserohilum turcicum* in *Zea mays*. J Fungi. 2023; 9(9). doi:10.3390/jof9090936
33. Manzar N, Kashyap AS, Goutam RS, Rajawat MVS, Sharma PK, Sharma SK, Singh HV. *Trichoderma*: Advent of Versatile Biocontrol Agent, Its Secrets and Insights into Mechanism of Biocontrol Potential. Sustainability. 2022; 14(19):12786. doi:10.3390/su141912786
34. Dutta P, Deb L, Pandey AK. *Trichoderma*- from lab bench to field application: Looking back over 50 years. Front Agron. 2022; 4:932839. doi:10.3389/fagro.2022.932839
AD, Khaloud MA, Zainul A. Application of powdered *Medicago sativa* L. Enhances eco-physiological output and protect against root rot fungi disease in okra and cowpea. Sci Hortic. 2024; 337:113458. doi:10.1016/j.scienta.2024.113458
20. Degani O, Gordani A, Dimant E, Chen A, Rabinovitz O. The cotton charcoal rot causal agent, *Macrophomina phaseolina*, biological and chemical control. Front Plant Sci. 2023; 14:1272335. doi:10.3389/fpls.2023.1272335
21. Airam E, Sanjuana C, Sepulveda E. Biocontrol of *Macrophomina phaseolina* Using *Bacillus amyloliquefaciens* Strains in Cowpea (*Vigna unguiculata* L.). Agronomy. 2022; 12(3):676. doi:10.3390/agronomy12030676
22. Marquez N, Giachero ML, Declerck S, Ducasse DA. *Macrophomina phaseolina*: General Characteristics of Pathogenicity and Methods of Control. Front Plant Sci. 2021; 12:634397. doi:10.3389/fpls.2021.634397
23. Wu D, Wang W, Yao Y, Li H, Wang Q, Niu B. Microbial interactions within beneficial consortia promote soil health. Sci Total Environ. 2023; 900:165801. doi:10.1016/j.scitotenv.2023.165801
24. Souto AL, Sylvestre M, Tölke ED, Tavares JF, Barbosa-Filho JM, Cebrián-Torrejón G. Plant-Derived Pesticides as an Alternative to Pest Management and Sustainable
35. Kumar K, Amaresan N, Bhagat S, Madhuri K, Srivastava RC. Isolation and Characterization of Trichoderma spp. for Antagonistic Activity Against Root Rot and Foliar Pathogens. Indian J Microbiol. 2012; 52(2):137-44. doi: 10.1007/s12088-011-0205-3.
36. Ben-David A, Davidson CE. Estimation method for serial dilution experiments. J Microbiol Methods. 2014; 107:214-21. doi:10.1016/j.mimet.2014.08.023
37. Jenssen AL, Kauserud H, Maurice S. High phenotypic variability in the wood decay fungus *Phellopilus nigrolimitatus*. Fungal Ecol. 2022; 59:100982. doi:10.1016/j.funeco.2020.100982
38. Oyewole BO, Olawuyi OJ, Odebode AC, Abiala MA. Influence of Arbuscular mycorrhiza fungi (AMF) on drought tolerance and charcoal rot disease of cowpea. Biotechnol Rep (Amst). 2017; 14:8-15. doi:10.1016/j.btre.2017.02.004
39. Chen L, Hao D, Dou K, Lang B, Wang X, Li Y, Chen J. Preparation of High Water-Soluble *Trichoderma* Co-Culture Metabolite Powder and Its Effects on Seedling Emergence Rate and Growth of Crops. J Fungi (Basel). 2023; 20;9(7):767. doi: 10.3390/jof9070767. PMID: 37504755; PMCID: PMC10381636.
40. Afouda L, Schulz D, Wolf G, Wydra K. Biological control of *Macrophomina phaseolina* on cowpea (*Vigna unguiculata*) under dry conditions by bacterial antagonists. Int J Biol Chem Sci. 2012; 6(6):5068-5077. doi:10.4314/ijbcs.v6i6.25
41. Persson L, Bødker L, Larsson-Wikström M. Prevalence and Pathogenicity of Foot and Root Rot Pathogens of Pea in Southern Scandinavia. Plant Dis. 1997; 81(2):171-174. doi:10.1094/PDIS.1997.81.2.171
42. Poveda J. *Trichoderma* as biocontrol agent against pests: New uses for a mycoparasite. Biol Control. 2021; 159:104634. doi:10.1016/j.biocontrol.2021.104634
43. Nieto-Jacobo MF, Steyaert JM, Salazar-Badillo FB, Nguyen DV, Rostás M, Braithwaite M, De Souza JT, Jimenez-Bremont JF, Ohkura M, Stewart A, Mendoza-Mendoza A. Environmental Growth Conditions of *Trichoderma* spp. Affects Indole Acetic Acid Derivatives, Volatile Organic Compounds, and Plant Growth Promotion. Front Plant Sci. 2017; 8:102. doi:10.3389/fpls.2017.00102
44. Guzmán-Guzmán P, Etesami H, Santoyo G. *Trichoderma:* a multifunctional agent in plant health and microbiome interactions. BMC Microbiol. 2025; 25:434. https://doi.org/10.1186/s12866-025-04158-2

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

45. Napolitano A, Senatore M, Coluccia S, Palomba F, Castaldo M, Spasiano T, Avino AG, Vitale A, Bonfante A, Sacco A, Ruocco M. Development and Evaluation of a *Trichoderma*-Based Bioformulation for Enhancing Sustainable Potato Cultivation. Horticulturae. 2024; 10(7):664. doi:10.3390/horticulturae10070664
46. Kumari P, Singh AK, Dewangan PK, Pankaj SC, Lakra AK. Effect of foliar application of nutrients on soybean. J Plant Dev Sci. 2017; 9(3):261-264.
47. Waleed A, Kelly DC, Ryota K, Ahmad M, Naveed A, Taqi R, Farhan I. The application of *Trichoderma* spp., an old but new useful fungus, in sustainable soil health intensification: A comprehensive strategy for addressing challenges. Plant Stress. 2024; 12:100455, https://doi.org/10.1016/j.stress.2024.100455.
48. Zin NA, Badaluddin NA. Biological functions of *Trichoderma* spp. For agriculture applications. Ann Agric Sci. 2020; 65(2):168-178. doi:10.1016/j.aoas.2020.09.003