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Research Article
Exploiting Potential of *Trichoderma harzianum* and *Glomus versiforme* in Mitigating *Cercospora* Leaf Spot Disease and Improving Cowpea Growth

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Abstract

Background and Objective: *Trichoderma* species are of utmost importance in agro-biotechnological applications because, in their interactions with plant hosts, they out-compete most pathogenic microorganisms. This study aimed at exploiting the potential of *Trichoderma harzianum* together with *Glomus versiforme* and its mutants, in inhibiting cowpea leaf spot rot induced due to *Cercospora canescens* infestation and improving agronomic growth parameter in a screen house experiment. **Materials and Methods:** The experiment was designed using single and co-inoculation of the bioagents: in all, eleven treatments were applied, consisting of Glom_verwild, Glom_ver30, Glom_ver60, Glom_ver90, Trich_h, Glom_verwild+Trich_h, Glom_ver30+Trich_h, Glom_ver60+Trich_h, Glom_ver90+Trich_h, Pathogen alone and control. Cowpea growth yield parameters and disease severity were assessed after 7 weeks. **Results:** The deployed treatments improved agronomic growth parameters substantially (p<0.05) relative to control. Glom_ver 60+Trich_h treatment exerted the highest agronomic growth improvement yield. In addition, the best reduction in the incidence and severity of cowpea leaf spot disease was obtained using $Glom\_{ver}$ 60+Trich_h. A significantly higher germination rate in seeding, confirms both inhibitory and growth improvement potency of the bio inoculants treatment. **Conclusion:** This study's findings confirmed the beneficial impacts of the treatment of seed and soil with dual *T. harzianum* and *G. versiforme*, in improving the immunity of cowpea to *Cercospora canescens* leaf spot infection and improve cowpea growth.

Key words: Microbial biocontrol agents, Cowpea leaf spot rot, improve cowpea growth, beneficial microbial resources, leguminous crops, agronomic growth improvement yield

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Achieving a sustainable and environmentally friendly agricultural production output in the world is a tasking challenge globally. These militating constraints are due to over-use of agricultural resources, urbanization issues, agricultural land encroachment and depletion, improper agrochemical usage, the challenges of harmful pests and microbial pathogens, environmental stress, change in climatic conditions, a burgeoning human population among others Doni *et al.*[1], Hannah *et al.*[2] and Berg.[3]

Vigna unguiculata L. Walp, popularly known as cowpea is an annual leguminous crop planted worldwide but mainly in the semi-arid region. It's a key component in farming systems in diverse ecological zones and serves as a source of nutritious human food, livestock feed, green manure and income source for smallholder farmers Lal[4].

One of the main constraints militating against the improved yield of cowpea production in most cowpea growing zones include; pests and microbial pathogens infestations both on the field and during postharvest storage, which account for up to 25 % of annual yield losses worldwide Afutu *et al.*[5] of these microbial pathogens, pathogenic fungal causes the most devastating crop losses at all stages in plants Dean *et al.*[6] *Cercospora* leaf spot (CLS), a fungal rot is associated with numerous plants, including a lot of economically important crops Bakhshi *et al.*[7].

CLS is associated with *Cercospora canescens* and *Pseudocercospora cruenta*, it affects cowpea production and leads to massive production loss Omoigui *et al.*[8].

Nevertheless, there are immense concerns over the inappropriate use of synthetic agrochemicals in the agro-food production process; these include over-utilization of non-renewable phosphate resources, nitrate pollution of groundwater, high fossil fuel energy consumption, as well as leaching or run-off and subsequent eutrophication. They also pose real risks to soil biodiversity and, in effect, potential food and feed supply Kliopova *et al.*[9] and Gianinazzi *et al.*[10].

Therefore, formulations based on microbes that are environmentally friendly, safe, biodegradable and environmentally sustainable seem to be better alternatives to conventional agrochemicals Pascual[11].

Trichoderma species are of utmost importance in agro-biotechnological applications because, in their interactions with plant hosts, they out-compete most pathogenic microorganisms within the root zones of plants using different mechanisms that include; secondary metabolites secretion, hydrolytic enzymes production, production of volatile organic compounds, phytohormones amongst others Yao *et al.*[12], Contreras-Cornejo *et al.*[13], Reino *et al.*[14], Zhang *et al.*[15].

Arbuscular mycorrhizal, well known as (AM) fungi belong to the Glomeromycota phylum, they have a widespread distribution in global ecosystems Bruns *et al.*[16].

(AM) fungi help in protecting plants against phytopathogens and also buffer against adverse environmental conditions Ren *et al.*[17] Therefore, maintaining an established and diverse AMF population and other beneficial microorganisms in the soil are of importance in achieving agricultural sustainability Olowe *et al.*[18].

However, little work has been done on exploiting the potential of using this eco-friendly, naturally occurring and sustainable fungi bio inoculants, in reducing cowpea *Cercospora* leaf spot disease.

This investigation is therefore focused on highlighting the potential of *Trichoderma harzianum* as single and co-inoculant of wild-type and mutated *Glomus versiforme* strains in controlling cowpea *Cercospora* leaf spot rot and to improve cowpea seedling growth.

MATERIALS AND METHODS

Experimental location: This pot experiment was conducted at the Research Institute responsible for Stored Product (NSPRI), Ilorin, Nigeria, using a screen house from 2016-2018.

Cowpea sample collection: The Research Farm of (NSPRI) and four other farm sites in Ilorin, Nigeria, was the source of cowpea leaves showing leaf spot symptoms. The signs are yellowish or with a yellow halo, others brown to purplish coloration symptoms on the cowpea leaves. The cowpea leaves were quickly processed to avoid contamination. Also, NSPRI was the source of the cowpea seeds.

Pathogens isolation and identification: Cowpea leaves were washed clean with water and then using a sterile blade cut in bits of 1 cm. The pieces were sterilized using a 0.5 % solution of sodium hypochlorite for 30 sec, rinsed in sterile water to remove surface contaminants. Potato Dextrose Agar (PDA), that was incorporated with 0.5 mg per liter of antibiotic was used for the growth of the leaves at ambient temperature for 7 d. After repeated sub-culturing, pure culture was obtained and stored on a slant. The identification of pure culture was done following Barnett and Hunter[19] protocol.

Pathogenicity test of the isolated pathogens: For pathogenicity test determination, this was done using the foliage spray method of spraying spore suspension of 2.5×10^4 conidia/mL of the isolated pathogens on cowpea leaves, while spraying the control cowpea leaves with ordinary water. This was followed by examination and comparison of the pathogen spore suspension sprayed foliage with the un-inoculated leaves, that received water spray. The pathogenic organism was re-isolated from cowpea leaves showing disease signs following Koch postulate. The re-isolated organisms were verified as the initial pathogen for confirmation.

Biological control agents (*T. harzianum* and *G. versiforme*): Bioagents used in carrying out this experiment was previously isolated and characterized in an earlier study Omomowo *et al.*[20]. The bioagents used in the study are:

- *Trichoderma harzianum*. (Trich_h) and *Glomus versiforme* ($Glom_{\_ver}$)
- $Glom_{\_ver}$ wild: wild type *G. versiforme*
- $Glom_{\_ver}$ 30: *G. versiforme* UV mutant, 30 min
- $Glom_{\_ver}$ 60: *G. versiforme* UV mutant, 60 min
- $Glom_{\_ver}$ 90: *G. versiforme* UV mutant, 90 min

Effects of dual inoculated *T. harzianum* and *G. versiforme* strains on *C. canescens*

Infested Cowpea: Steam sterilized soil was used for planting in this experiment. The soil was left for 1 week to enable the escape of volatile toxic substances created while sterilizing. Then, each pot received 300 g of soil, used in growing cowpea plants in insect screening experiments Mwangi *et al.*[21].

Twenty five gram inoculum of the bioinoculant treatments Glom_ver wild, Glom_ver 30, Glom_ver 60 and Glom_ver 90; were inoculated into the pot to a depth of about 0-5 cm, then cowpea seeds planted, followed by another 25 g inoculum addition. The application of *T. harzianum* inoculant was done using 60 spores per gram of soil in each pot. The control pot was not inoculated with the bioinoculant agents.

Influence of co-inoculation with *T. harzianum* and *G. versiforme* on incidence and severity of Cowpea *C. canescens* rot: The percentage disease incidence was counted 15 days after sowing for seedling-emergence, 45 days for post-emergence of leaf spot disease. The number of leaf spot disease plants and survived/healthy plants were assessed at harvest 49 days (7 weeks). The percentage severity and incidence of disease were determined using the following formula:

$$\text{Incidence of disease} = \frac{\text{Infected plants number}}{\text{Total of observed plants}} \times 100$$

Also, estimation of disease severity was done using 0-5 scale Lee *et al.*[22] where: 0 = healthy plant, 1 = 1-25% of the leaves were diseased, 2 = 26-50% of the leaves were diseased, 3 = 51-75% of leaves were diseased, 4 = 76-99% leaves diseased, 5 = Dead or inactive plant:

$$\text{Calculation of severity of disease} = \frac{\sum(\text{Infected plants} \times \text{Degree of infection})}{\text{Total plants tested} \times \text{Highest degree infected}} \times 100$$

Percentage colonization determination: The first step is to wash cowpea roots thoroughly with clean water and then cut them into 1 cm pieces each. The different processes included staining, destaining and acidification Koske and Gemma[23].

Then observation of roots that was stain, using a microscope with magnifications of 10, 40 and 100. Colonization percentage estimation was then determined Mcgonigle *et al.*[24].

$$\text{AM fungi colonization percentage} = \frac{\text{Infected number of mycorrhiza}}{\text{Root segments observed number}}$$

Effects of soil bioinoculant treatments using *T. harzianum* and G*. versiforme* on Cowpea growth improvement: At harvest which was 7 weeks post-seed sowing, the cowpea plant was processed to assess the agronomic yield parameters. This entails the separation of roots, drying at 70°C (48 h), determining and recording the area of the leaf, height of plant, root, stem and shoot.

Effects of seeds treatment with *T. harzianum* and *G. versiforme* on *C. canescens* infested Cowpea: Seeds of cowpea were sterilized using sodium hypochlorite at 5%, thereafter washed with sterile water and aseptically air-dried. Surface sterilized cowpea seeds were inoculated with 2 mL spore suspension of the bioagents (25 g of spore suspensions of *G. Versiforme* and 4×10^8 conidia mL^{-1} of *T. harzianum*), mixed with 2% carboxyl methylcellulose (CMC) solution as a sticker and shaken slowly for 5 min on a magnetic stirrer Hashem *et al.*[25].

Seven cowpea seeds were sown in each pot that was infested using *C. canescens*. Bioagents were seed inoculated and the control pot was without any inoculant treatment. Agronomic growth parameter data was recorded and this includes measurement of shoot length and length of the root. The percentage germination rate, weight of seedlings, the normal, abnormal and diseased seedlings, as well as seeds that are dead or did not germinate, were also estimated.

$$\text{Vigor index (\%)} = (\text{Average length of shoot} + \text{Average length of root}) \times \text{Germination (\%)}$$

The experimental treatments used are:

Treatment 1 : Cowpea seed+*C. canescens*+Glom$_{_ver}$ Wild
Treatment 2 : Cowpea seed+*C. canescens*+Glom$_{_ver}$ 30
Treatment 3 : Cowpea seed+*C. canescens*+Glom$_{_ver}$ 60
Treatment 4 : Cowpea seed+*C. canescens*+Glom$_{_ver}$ 90
Treatment 5 : Cowpea seed+*C. canescens*+Trich_honly
Treatment 6 : Cowpea seed+*C. canescens*+Trich_h +Glom$_{_ver}$ Wild
Treatment 7 : Cowpea seed+*C. canescens*+Trich_h +Glom$_{_ver}$ 30
Treatment 8 : Cowpea seed+*C. canescens*+Trich_h +Glom$_{_ver}$ 60
Treatment 9 : Cowpea seed+*C. canescens*+Trich_h +Glom$_{_ver}$ 90
Treatment 10 : Cowpea seed+*C. canescens*
Treatment 11 : Cowpea seed only (Control)

This experimental treatment setup was replicated 5 times.

Statistical analysis: Data analysis was done using analysis of variance and ($p\leq 0.05$) was considered significant. Least square difference (LSD) was used in comparing means and this was done with version 17.0 SPSS statistical software.

RESULTS

Isolating and identifying pathogens from the Cowpea plant: Isolation and identification of fungus specie from cowpea plants that had shown leaf spot disease symptom and subjected to pathogenicity test yielded the fungus *Cercospora canescens*. Though organisms isolated were of like genera but different species; the pathogenicity test affirmed *C. canescens* as the pathogen responsible for leaf spot infestation (Table 1).

G. versiforme* and *T. harzianum* bio inoculants effects on growth parameters of *C. canescens* soil infested *Vigna unguiculata: At harvest, 49 days (7 weeks) after sowing, bio inoculants influence on cowpea seedling height, leaves number, the dry and fresh root/shoot weight, as well as area index of the leaf, was significant. Synergistic inoculation using Glo$_{_ver}$ 60+Trich-h performed best, with 37.3 cm plant height, 18.4 number of leaves, the bio inoculants significantly influenced height, leaves number, fresh shoot weight -11.96 g, fresh root weight -3.46 g, dry shoot weight -7.30 g, dry root weight -1.42 g and percentage mycorrhizal colonization -75.1 cm^2. The control results were 30.1 cm, 15.4, 6.41 g, 1.34 g, 3.53 g, 0.83 g and 38.7 cm^2, respectively. This is illustrated in (Table 2).

Table 1: The incidence and pathogenicity of *Cercopsora* isolate from cowpea plants displaying leaf spot disease obtained from investigation sites

Code of isolates	Isolate origin	Aspects of colonies	Mycelia diameter (mm)	Number of isolates	Pathogenicity	Species
L1	Baba Ode	Cotton Like	58	1	-	*Cercospora* sp.
L2	Oko Olowo	Cotton like	50	1	+	*Cercospora canescens*
L3	NSPRI	Flat	43	2	-	*Pseudocercospora cruenta*
L4	Ita Elepa	Cotton	51	1	-	*Cercopora* sp.

+: Symptoms, -: No Symptoms, Values are means in replicates

Table 2: *G. versiforme* and *T. harzianum* bio inoculants effects on growth parameters of *C. canescens* soil infested cowpea

Treatment	Plant Height (cm)	Leaves number	Leaf area index (cm^2)	Fresh weight (g) Shoot	Fresh weight (g) Root	Dry weight (g) Shoot	Dry weight (g) Root	Mycorrhizal colonization (%)
L2	24.5	9.2	35.3	5.63	1.22	3.40	0.33	--
L2+Glom$_{_ver}$ wild	31.2	15.3	37.3	7.83	2.16	3.96	0.71	75.40
L2+ Glom$_{_ver}$ 30	29.2	16.1	40.7	6.45	2.31	4.26	0.61	76.60
L2+Glom$_{_ver}$ 60	30.6	17.2	53.8	8.47	2.43	4.71	0.75	80.10
L2+Glom$_{_ver}$ 90	31.6	16.3	39.3	8.93	2.03	4.93	0.62	77.30
L2+Trich-H	32.4	17.5	55.6	9.50	2.41	5.10	0.78	-
L2+Glom$_{_ver}$ wild+Trich_h	32.4	17.8	58.8	10.21	2.63	6.54	0.94	63.90
L2+Glom$_{_ver}$ 30+Trich_h	34.1	18.1	60.6	10.17	2.43	6.43	0.85	65.75
L2+Glom$_{_ver}$ 60+Trich_h	37.3	18.4	75.1	11.96	3.46	7.30	1.42	67.45
L2+Glom$_{_ver}$ 90+Trich_h	33.7	18.0	67.7	10.8	2.37	6.93	0.97	60.15
Control	30.1	15.4	38.7	6.41	1.34	3.53	0.83	--
Mean	31.5	16.7	51.3	8.7	2.3	3.9	0.8	51.3
SEM	0.41	0.43	1.84	0.26	0.07	0.19	0.04	4.39
LSD (0.05)	0.25	2.64	0.70	0.26	1.91	0.06	0.18	0.84
p-level	***	***	***	***	***	***	***	***

Values represent five replicates, ***: Mean value was significant at probability value of $p<0.05$, SEM: Standard mean error, L2: *C. canescens*, Trich_h: *T. harzianum*, Glom$_{_ver}$: *Glomus versiforme*, 30, 60, 90: UV mutants for 30, 60 and 90 min

Table 3: Impacts of synergetic inoculation using *T. harzianum* and *G. versiforme* wild type/mutated strains on disease severity of leaf spot induced by *C. canescens* infested soil on *Vigna unguiculata*

	Disease incidence (%)			
	At the seedling stage (3 weeks)		At Mature plant (7 weeks)	
Biocontrol soil preparation	Pre-emergence	Post-emergence	Leaf spot	Healthy seedling
$Glom_{\_ver}$ wild	20.0	16.6	16.4	47.0
$Glom_{\_ver}$ 30	23.1	16.9	16.9	43.1
$Glom_{\_ver}$ 60	15.6	12.0	10.0	59.4
$Glom_{\_ver}$ 90	20.0	13.3	13.3	53.4
Trich_h	15.8	14.0	10.0	60.2
$Glom_{\_ver}$ wild+Trich_h	13.3	10.0	13.3	63.4
$Glom_{\_ver}$ 30+Trich_h	13.3	13.3	10.4	60.0
$Glom_{\_ver}$ 60+Trich_h	0.0	6.3	6.3	87.4
$Glom_{\_ver}$ 90+Trich_h	12.0	10.7	11.3	66.0
Control	23.8	20.0	24.2	32.0
Mean	15.7	13.2	14.4	57.2
SEM	0.93	0.53	1.23	2.02
LSD (0.05)	0.27	0.38	10.56	1.56
P-level	***	***	Ns	***

***: Mean squares significant at p<0.05, Ns: Mean squares non-significant at (p>0.05), SEM: Standard mean error

Table 4: Influence of *T. harzianum* inoculated seeds that are co-inoculated with *G. versiforme* mutated strains and wild type on cowpea leaf spot infestation initiated by *C. canescens*

Treatment	Germination (%)	Shoot length (cm)	Root Length (cm)	Vigor index	Abnormal seedling	Diseased seedling	Dead seeds	Weight of seedlings (g)
$Glom_{\_ver}$ wild	72.0	4.7	6.9	830.0	2.10	1.60	2.68	9.82
$Glom_{\_ver}$ 30	74.0	4.9	7.4	916.0	2.00	1.50	2.45	10.10
$Glom_{\_ver}$ 60	83.0	5.8	8.5	1189.0	1.60	1.10	1.73	10.50
$Glom_{\_ver}$ 90	78.0	5.3	7.9	1030.0	1.80	1.40	2.08	10.20
Trich_h	84.0	5.8	8.7	1221.0	1.40	1.30	1.69	10.48
$Glom_{\_ver}$ wild+Trich_h	86.0	6.3	8.9	1309.0	0.00	0.00	1.48	10.79
$Glom_{\_ver}$ 30+Trich_h	88.0	6.9	9.3	1429.0	0.00	0.00	1.36	10.86
$Glom_{\_ver}$ 60+Trich_h	94.0	7.4	10.4	1677.0	0.00	0.00	1.00	11.24
$Glom_{\_ver}$ 90+Trich_h	91.0	7.1	9.8	1543.0	0.00	0.00	1.20	11.10
Control	70.0	4.1	6.4	739.0	5.20	4.36	4.20	5.34
Mean	82.0	5.9	8.4	1188.0	1.46	1.12	1.92	10.18
SEM	1.17	0.15	0.18	2.36	0.23	0.18	0.15	0.25
LSD (0.05)	3.85	0.19	0.31	4.96	0.70	0.58	0.28	0.25
p-level	***	***	***	***	***	***	***	***

Values represent five replicates, ***: Mean value was significant at probability value of p<0.05, SEM: Standard mean error

Impacts of synergetic inoculation using *T. harzianum* and *G. versiforme* wild type / mutated strains on disease severity of leaf spot induced by *C. canescens* infested soil on *Vigna unguiculata*: Results of synergetic inoculation with the bioagents on *Cercospora* leaf spot disease incidence in cowpea grown on soil infested with *C. canescens*, led to a significant reduction in disease severity at both pre and post seedling emergence. Glo_{ver} 60+TH (6.30 %) gave the least percentage of disease incidence and the highest percentage of healthy seedlings. This was followed by Glo_{ver} 90+TH (11.3%) and (66%). This is illustrated in Table 3.

Effect of seed treatments with *T. harzianum* that are co-inoculated with *G. versiforme* mutated strains and wild type on Cowpea leaf spot infestation initiated by *C. canescens*: The influence of using bioagents seed priming on leaf spot disease occurrence and severity caused by *C. canescens*; was a positively beneficial effect (Table 4). There was an improvement in cowpea growth and a reduction in seedling disease. The germination rate was (94%) for Glom_ver 60+Trich_h bioinoculant and (91%) for Glom_ver 90+Trich_h. The bioinoculant Glom_ver 60+Trich_h vigor index value was (1677.00), while the value for Glom_ver 90+Trich_h was (1543.00) and the lowest value was obtained in the control (739.00).

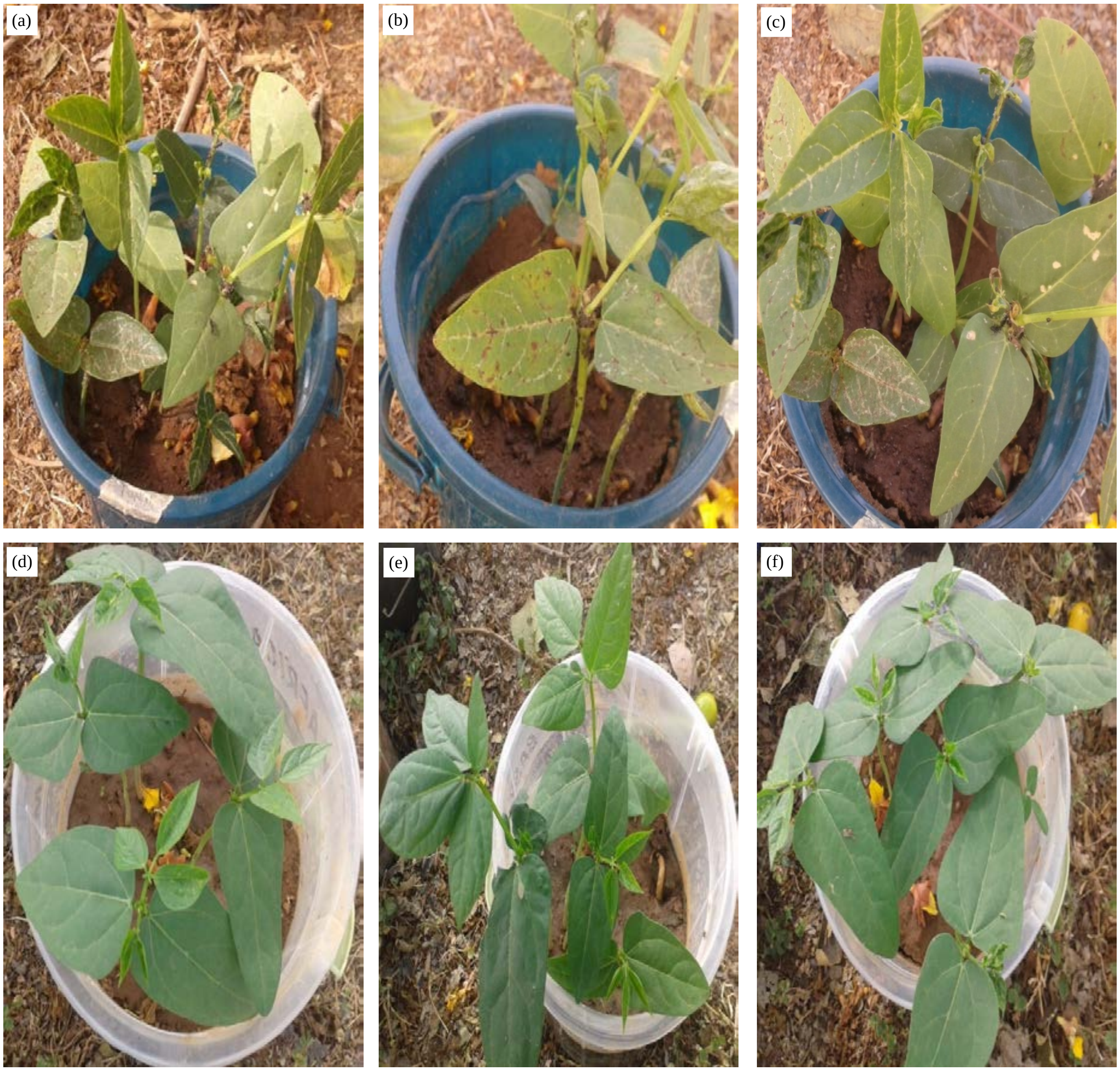

Fig. 1(a-f): Cowpea seedlings at harvest 49 days (7 weeks) of soil bioinoculant treatments, C: Control pot: L2: *Cercospora* leaf spot infested pot; Glom$_{_ver}$ wild+Trich_h: Wild type of *Glomus versiforme* and *Trichoderma* pot, Glom$_{_ver}$ 30+Trich_h: *G. versiforme* UV induced for 30 min and *Trichoderma* pot, Glom$_{_ver}$ 90+Trich_h: *G. versiforme* UV induced for 90 min and *Trichoderma* pot, Glom$_{_ver}$ 60+Trich_h: *G. versiforme* UV induced for 60 min and *Trichoderma* pot, (a) Control, (b) L2, (c) Glom$_{_ver}$wild+Trich_h, (d) Glom$_{_ver}$ 30+Trich_h, (e) Glom$_{_ver}$90+Trich_h and (f) Glom$_{_ver}$ 60+Trich_h

Figure 1 indicated that the bioinoculant treatment Glom_ver 60+Trich_h performed best morphologically.

DISCUSSION

This study aimed at exploiting the potential of using *T. harzianum* as a single inoculant and as mixed-inoculant with *G. versiforme* mutated strains and its wild type to suppress *C. canescens* infestation in cowpea, as well as improve growth. These findings highlighted the effectiveness of these bioagents in suppressing the disease severity incidence of cowpea leaf spot and it also led to the improvement in the growth parameter of inoculated cowpea plant when compared with the control.

The results obtained, showed that the microbial inoculants led to an improvement in cowpea growth and also reduce the occurrence and severity *C. canescens* induce leaf spot infestation. There was a significant ($p<0.05$) increase in the height, root weight, weight of shoot (both dry and wet) due to the bioinoculant treatment.

Our findings on using the synergetic influence of the bioagents, indicated better significant performance due to the mixed inoculation, compared to single inoculant. This indicated that there were positive synergistic effects of the co-inoculation of the two bioagents on the cowpea plant.

Previous studies highlighting the beneficial influence of mycorrhizal fungi, in synergy or co-inoculation with other bioagents on crops have been reported by Ma *et al.*[26]

The synergistic influence of the bioagents might be responsible for the wider spectrum of beneficial influence in terms of growth improvement and pathogen inhibitory activity that was observed in cowpea plants in this experiment.

The findings, also indicated that seeds treatment with the biocontrol agent gave the highest rate of germination (94.0%), while control was (70.0%).

Also, the biocontrol agent coated seeds improved significantly, the shoot and root length of the cowpea plant. These findings agrees with work done by different researchers that affirmed the potency of using both mycorrhizal fungi and other growth-promoting microbial species to positively influence the growth, vigor, vitality and disease resistance capability of plants by using different mechanisms Bowles *et al.*[27] Pérez-de-Luque *et al.*[28].

Also, findings in the course of the experiment indicated a reduction in mycorrhizal colonization efficiency when co-inoculants of *T. harzianum* with *G. versiforme* were applied, compared to higher efficiency, when *G. versiforme* was used alone.

These findings are consistent with the results of Tchameni *et al.*[29] that reported a reduction in colonization efficiency of AMF when co-inoculated with other microbial resources.

This could be the plausible explanation for variation in results obtained for disease severity reduction and improvement in cowpea growth, attributable to bioagents inoculation.

The results obtained in this present study confirmed previous studies that affirmed that arbuscular mycorrhizal fungi are widely distributed and they colonize roots of most plants. Also, colonization by (AM) fungi leads to positive changes in the morphology, physiology and nutritional uptake in plants that improve their growth, vigor and vitality. This is achieved through the architectural modification of plant roots to enhance access to needed nutrients and water Thirkell *et al.*[30].

Earlier report ascribed (AM) fungi as plant growth promoters through their ability to improve the structure of the soil, enhance water uptake and mineral nutrients by plant roots Nafady *et al.*[31] They are also known to help in protecting plants from phytopathogens Sharma and Sharma[32].

Mycorrhizal positively influences the growth of planted crops by enhancing the structure of the soil and also improving the nutrients that are available to plants Berruti *et al.*[33], Cobb *et al.*[34]

Furthermore, the biocontrol agent *Trichoderma* is a well-known unique genus in the phylum ascomycetes due to its resourcefulness in adapting to diverse ecological systems and situations. They are widely applied as biological fungicides in modern agriculture due to the ability to enhance plant defense against pathogenic organisms and also, due to their plant growth improvement, vigor and vitality enhancement, as well as their influence on abiotic stress tolerance capability in plants Mendoza-Mendoza *et al.*[35]. They can antagonize microbial pathogens by deploying a process known as mycoparasitism Kubicek *et al.*[36] All these highlighted reports are in agreement with the results that were obtained in this present investigation.

However, an additional research study that will focus on improving the bioinoculant agents to withstand on-field experimental constraints should be done in the future. This should be done along with efforts to optimize the use of readily available and cheaper substrates for bioformulation of the bioinoculant agents.

CONCLUSION

Findings from this study confirmed the potential of *T. harzanium* when co-inoculated with wild type and mutant strains of *G. versiforme* in serving as biocontrol agents in controlling cowpea *Cercospora* leaf rot and also improving cowpea seedling growth attributable to both soil and seeds bioinoculant treatments.

SIGNIFICANCE STATEMENT

This study discovers that the synergistic influence of co-inoculating *T. harzanium* with wild type and mutated strains of *G. versiforme was* effective in controlling cowpea *Cercospora* leaf rot and also improved cowpea seedling growth. This study will encourage researchers to bioprospect and explore different ecological niches for beneficial fungal strains that can be used as bioinoculants to inhibit disease severity and improve the growth of planted crops. Thus, an addition to the theory on synergetic influence and potency of bioinoculants *T. harzianum* and wild type and mutated strains of *G. versifome* was arrived at in this study.

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