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Influence of Arbuscular mycorrhiza fungi (AMF) on drought tolerance and charcoal rot disease of cowpea

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ABSTRACT

The influence of Arbuscular mycorrhiza fungi (AMF) (*Glomus deserticola* and *Gigaspora gigantea)* were evaluated on drought tolerance and charcoal rot disease of cowpea genotypes: IT90K-277-2, IT84S-2246-4 and IT06K123-1. IT90K-277-2 and IT84S-2246-4 were sown in 3 kg of sterilized soil for drought experiment with five treatments. Treatment was established thirty days after germination with inoculation of *G. deserticola*, the mycorrhizal treated cowpea withstand the water stress and produced high yield. Biocontrol experiment had 2 kg sterilized soil potted into bags with cultivars IT90K-277-2 and IT06K123-1, fourteen treatments were established with soil drenched before planting and simultaneous inoculation. Soil drenched with AMF before planting and inoculation of *M. phaseolina* after 10 days of germination recorded higher growth parameters, while the simultaneous inoculated plant was the most effective in reducing disease severity. However, simultaneous treatment of *G. deserticola*, *G. gigantea* and *M. phaseolina* were most effective for both growth parameters and reduction of disease severity.

1. Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is one of the most important drought-resistant leguminous food crops grown for its foliage and grain in tropical and sub topical Saharan Africa regions of the world particularly in Nigeria [1,2]. There are variations among cowpea genotypes in their drought tolerating potentials [3,4]. Turk et al. [5] cited in Ahmed and Suliaman [6] affirmed that cowpea is highly sensitive to water stress during the flowering and pod-filling stages. Though, there are limited information on the response of the crop to drought at different stages of growth. According to Ajibade and Amusa [7], its cultivation in humid agroecologies of South West Nigeria is also faced with several pests and diseases such as brown blotch, anthracnose , *cercospora* leaf spot, *choaniphora* pod rot, false smut and web blight, charcoal rot and *sclerotium* stem blight of which *Macrophomina phaseolina* is included. The effect of field diseases has led to reduction in yield of cowpea.

Macrophomina phaseolina (Tassi) Goidanich, is one of the most destructive plant pathogens in the tropics and subtropics causing diseases in a wide range of host plant [8,9]. The pathogen was detected in Chile in 1983 in *Pinus radiata* D., Don nurseries in the Bío-Bío Region In the last few years, dissemination of the pathogen had been detected from the nurseries to the plantations through asymptomatic plants. Mortality of plant would be observed in the first years of the plantation when they are predisposed to conditions such as hydraulic stress and high soil temperatures [10]. *M. phaseolina* is a saprophyte that survives in the soil due to micro-sclerotinia formation which is pseudoparenchymal tissue masses resistant to adverse environmental conditions [11].

The most successful control strategy for charcoal root rot in forest nurseries was soil fumigated with methyl bromide [10]. Although, some of the problems associated with the application of chemicals include high cost, environmental pollution, breaking up the ecological balance of the soil, as well as the destruction of the ozone layer [12,13]. Biological control has been considered as an alternative selective method to control this disease [14]. Several researchers had focused on antagonist microbes such as *Bacillus, Pseudomonas, Streptomyces, Trichoderma, Penicillium, Rhizopus, Aspergillus* but there are limited studies on the adoption of mycorrhiza fungi biotechnology as control strategy Olawuyi et al., 2014a,b.

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Mycorrhiza association is a symbiotic non-pathogenic relationship between plant roots and fungal hyphal [15,16]. In this relationship, the fungi obtain carbon compounds and other nutritional requirements from the symbiotic plant roots, and in return, supply the plant with most of the immobile mineral elements such as Nitrogen (N), Phosphorus (P), Potassium (K), Calcuim (Ca), Copper (Cu) and Zinc (Zn) from the soil solution. The importance of mycorrhiza association in both agricultural and ecological systems had earlier been widely recognised [17,15]. The increased plant growth by VAM association is usually due to increased mineral elements uptake by the hyphae from the soil [18], Olawuyi et al., 2012), improved water relations and pest resistance of host plants [19,20], plants tolerance to a variety of abiotic stresses [21], increased resistance to soil pathogens [22,23,24], 2013). Mycorrhizae can also resist drought in many plants under stress conditions therefore the plants infected with VA mycorrhizae are less likely to wilt under drought affected conditions [25,26,27].

Therefore, the study aimed at investigating the influence of AMF (*Glomus deserticola*) on water stress in cowpea and established the effect of AMF (*Glomus deserticola and Gigaspora gigantea*) on *Macrophomina phaseolina* causing charcoal rot in cowpea.

2. Materials and methods

2.1. Experimental location and research design

Planting of cowpea seeds was done at the screen house, while the isolation and identification were carried out at the research farm and pathology laboratory respectively in the Department of Botany, University of Ibadan. complete randomized design was used for the two experiments with three replicates.

2.2. Source of seed samples and inocula

Cowpea seeds were collected from the germplasm unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Seeds varieties include: drought susceptible (IT90K-277-2 and IT84S-2246-4), and pathogen susceptible (IT06K-123-1 and IT90K-277-2). Rhizosphere soil of cowpea where the pathogenic organism was isolated, was also collected from IITA, while Arbuscular mycorhiza fungi (*Glomus deserticola* and *Gigaspora gigantea*) were obtained from the Department of Botany, University of Ibadan.

2.3. Media preparation, isolation of organism and slide preparation

20 g of PDA dissolved in 500mls of distilled water was prepared and autoclaved at 121 °C for 15mins.The prepared PDA was allowed to cool to 45 °C, while streptomycin was added to inhibit the growth of bacteria and the solution was gently swirled to obtain a homogeneous mixture. 2 g of soil sample was serially diluted using dilution factors of 10^{-1}, 10^{-2},10^{-3},10^{-4},$10^{-5}$and 10^{-6}, 1 ml is taken each from 10^{-1}, 10^{-3} and 10^{-6} inoculated into prepared Potato dextrose Agar media (PDA) in Petri dishes using pour plate method, Incubation was done at room temperature for 5–7 days. Mixed culture was sub-cultured to obtain pure culture of the pathogen. Stock culture was obtained from the pure culture of the organism which were prepared on PDA slant and stored at about 4 °C in the refrigerator. Slides of pure cultures obtained were prepared using sterilized needle to pick mycelia growth of the organism onto the slide surface. With the aid of the needle and addition of drop of sterile distilled water, the mycelia were properly dislodged. A drop of lacto phenol in cotton blue stain was then added to aid clarity when viewed under microscope.

2.4. Morphological identification and characterization of fungi isolates

The identification and classification of these structures were compared to that of Domsch et al. [28] in compendium of soil fungi.

The electronic microscope was used in viewing the prepared slides for the various morphological characters ranging from micro conidia, macro conidia, conidia shape and hyphal arrangement.

2.5. Soil sterilization and seed viability test

The soil (sandy loam soil) used in conducting the research work was collected from the Research farm of the Department of Botany, University of Ibadan. Sterilization of the soil was done using electric soil sterilizer and allowed to cool before packed into pottling bags.

The test for viability was done using the method of, in which 10 seeds were sown per bag. Each variety was sown in triplicates and the percentage germination was obtained from the formulae below;

$$\%\text{germination} = \frac{\text{No. of germinated seed}}{\text{Totalno of seed planted}} \times 100$$

2.6. Seed planting and pathogenicity test

A sterilized sandy loam soil weighing 2 kg and 3 kg potted into pottling bags were used for disease resistance and drought tolerance experiments in varieties of cowpea respectively. Ten seeds were planted per bag and then thinned to two per bag after three weeks of growth.

The pathogenicity test was carried out using modified method of Ahmed et al. [51]. The mycelia suspension of *Macrophomina phaseolina* isolate was prepared by blending ten 5 mm mycelia disc from 10 to 15 day-old culture of the fungus in 100 ml of sterile distilled water using warring blender. 5 ml of the inoculum mycelia suspension was injected round the wounded region of the lower stem of the seedlings after 10 days of germination.

Reactions of cowpea varieties to drought as influenced by Glomus deserticola

Ten seeds each of the drought susceptible cowpea varieties (IT90K-277-2 and IT84S-2246-4) were sown separately in 3 kg sterilized sandy loam soil. Germination of seeds was observed after 4 days of planting. The plants were thinned to two seedlings per plant after 14 days of emergence. Five treatments were established on the two cowpea varieties after 30 days of germination with inoculation of 5 ml mycelia suspension of *Macrophomina phaseolina* and 10 g (32spores) of *Glomus deserticola*. These treatments included;

T1- cowpea alone + watering
T2- cowpea alone + water stressed
T3- cowpea infested + AMF (*Glomus deserticola*) + water stressed
T4- cowpea infested + AMF *(Glomus deserticola*) + watering
T5- cowpea infested + AMF (*Glomus deserticola*) + *Macrophomina phaseolina* + water stressed

The rating scale for drought was from 1 to 5 according to the procedures of Auge [29], Al-Karaki et al. [30] and Olawuyi et al. Olawuyi et al. (2011b,c).

1. Excellent, normal plant growth, number of plant less than 10% water stress.
2. Good, slight drought but noticeable stunting, slight reduced leaf and number of leaf 11–25%

3. Moderate stunting, moderate leaf reduction, number of plant 26–50%
4. Poor, heavy drought, severe stunting, reduction in leaf, leaf wilting and rolling, number of plant 51–75%
5. Too poor, very heavy drought, definite leaf with extensive and conspicuous stunting, leaf wilting, leaf rolling, severe reduction in stem diameter, severe damage and premature death.

2.7. Disease resistance of M. phaseolina by mycorrhiza fungi

2.7.1. Planting and inoculation methods

Some of the potted soil used was drenched with the Arbuscular mycorrhiza fungi (*Glomus deserticola* and *Gigaspora gigantea*) before sowing of seeds. 10 seeds of each of the pathogen susceptible cowpea varieties were sown in 2 kg sterilized sandy loam soil. Germination of seeds was observed after 4 days of planting. The plants were thinned to two seedlings per pot after 7 days of emergence. Fourteen treatments applied after 10 days of germination included;

T1- cowpea (control)
T2- cowpea + *Glomus deserticola* (drench)
T3- cowpea + *Glomus deserticola*
T4- cowpea + *Gigaspora gigantea* (drench)
T5- cowpea + *Gigaspora gigantea*
T6- cowpea + *Glomus deserticola* + *gigaspora gigantea* (drench)
T7- cowpea + *Glomus deserticola* + *Gigaspora gigantea*
T8- cowpea + *Macrophomina phaseolina*
T9- cowpea + *Glomus deserticola* + *M. phaseolina* (drench)
T10- cowpea + *Glomus deserticola* + *M. phaseolina*
T11- cowpea + *Gigaspora gigantea* + *M. phaseolina* (drench)
T12- cowpea + *Gigaspora gigantea* + *M. phaseolina*
T13- cowpea + *Glomus deserticola* + *Gigaspora gigantea* + *M. phaseolina* (drench)
T14- cowpea + *Glomus deserticola* + *Gigaspora gigantea* + *M. phaseolina*

Application of *Glomus deserticola* and *Gigaspora gigantea* were done in two forms; some pots of soil were drench with 10 g of AMF before planting, while the other treatments was applied after 10 days of plant emergence at a radius of 20 cm from the root of the cowpea plant. Simultaneously with the mycellial suspension of the pathogen (*M. phaseolina*) which was inoculated around wounded region of the lower stem of the seedlings after 10 days of germination.

2.8. Determination of disease incidence and severity

2.8.1. Disease incidence

The percentage disease incidence of infected cowpea seedlings was estimated as described by Persson et al. [31].

$$\%\ \text{Disease incidence} = n/N \times 100$$

Where n = number of plant showing diseased symptoms with at least a brown coloration of the stem and N = Total Number of sample used.

2.9. Disease severity

This was done using the method of Persson et al. [31] as modified by Ahmed et al. [51].

0 = Healthy plant without any visible symptoms
5 = Discolouration of less than 5 mm of the root system
10 = Discolouration of about 20 mm of the root system
25 = About 5% of the root system discoloured
50 = The whole root system discoloured but no symptom on the epicotyls or leaves
75 = The whole root system as well as epicotyls discoloured with the lower leaves wilted
100 = Dead plants

2.10. Data collection and statistical analysis

Agronomic data collected at 7 days intervals include; plant height, leaf area, stem girth, number of leaves per plant, number of pod per plant and pod weight per plant were taken at the yield stage. Response of plants to drought tolerance, disease incidence and disease severity were also taken.

The leaf area was calculated using the method described by Jolaoso [32].

$$\text{Leaf length} \times \text{leaf width} \times \text{correction factor}$$

Where correction factor = 2.7

Statistical analysis of data were analyzed by ANOVA using SPSS version 20, while treatment means were separated using Duncan multiple range test at 5% level of probability.

3. Results

The growth response of the cowpea cultivars (IT190k-277-2 and IT84S-2246-4) to drought stress shows no significant difference on the growth parameters; plant height, leaf area and numbers of leaves at the early stage of treatment (1WAT and 2WAT), while at 3WAT and 4WAT the number of leaves of the two cultivars subjected to water stress were significantly different ($p < 0.05$), where treatment with *Glomus deserticola* (AMF) recorded higher numbers of leaves 37.33b, 36.67ab for IT190k-277-2 and 42.00b, 36.67b for IT84S-2246-4 at 3WAT and 4WAT respectively. It is also observed at the maturing stage (pod filling) 4WAT that AMF has influence on the leaf area of the cultivar IT84S-2246-4 with significant difference between the C + AMF + WS (200.48ab) and C + WS (142.17b) (Table 1).

The result of the response of cowpea cultivars to different growth stages at 7, 14, 21, 28 days before treatment and days after treatments is shown in Table 2. The observation during the before-treatment stage indicates that the growth parameters; plant height, leaf area and numbers of leaves per plant were significantly different ($p < 0.05$) in both cowpea cultivars IT90K-277-2 and IT84S-2446-4, except in cultivar IT90K-277-2 with the leaf area of 82.41 cm^2 and 80.35 cm^2 which are not significantly different ($p > 0.05$) at 14 and 21 days before treatment respectively. While at days after treatment a significant difference ($p > 0.05$) is observed at 7DAT for plant height and numbers of leaves for the two cultivars.

The mean square effect of treatment, replication and growth after treatment is shown in Table 3. At before treatment stage IT90K-277-2 was not significant for plant height, leaf area and numbers of leaves per plant in both treatment and replicate levels, but highly significant ($p < 0.05$) for days before treatment. In IT84S-2446-4, the treatment effect was highly significant for plant height and numbers of leaves, but significant ($p < 0.05$) for leaf area at before treatment. The leaf area and numbers of leaves per plant were not significant at the replicate level, but highly significant for plant height. The effect of days before treatment was highly significant for all the characters. At after treatment stage, IT90K-277-2 did not produce significant effect for plant height and leaf area at both treatment and replicate levels, but significantly different for numbers of leaves per plant. There was significant effect for days after treatment in plant height and numbers of leaves, but non- significant for leaf area. Again, the height of plant

Table 1
Growth response of cowpea cultivars; IT90K-277-2 and IT84S-2246-4 to *Glomus deserticola, Macrophomina phaseolina* and their treatments.

	Treatments	Plant height (cm)	Leaf area (cm^2)	No of leaves	Plant height cm	Leaf area (cm^2)	No of leaves
Initial Reading	C + W	44.40a	161.61a	19.33a	36.90b	160.57a	14.00b
	C + WS	38.20a	141.75a	16.00a	39.83ab	215.53a	18.00a
	C + AMF + WS	40.53a	174.73a	16.00a	39.67ab	203.57a	16.00ab
	C + AMF + W	40.23a	149.16a	15.00a	43.17a	219.58a	16.00ab
	C + AMF + M + WS	40.40a	145.44a	15.00a	36.80b	216.11a	18.00a
1 WAT	C + W	61.37a	222.39a	26.00a	49.00a	227.84a	20.67a
	C + WS	51.13ab	202.75a	21.00a	53.60a	232.92a	24.00a
	C + AMF + WW	50.30ab	223.24a	23.00a	57.23a	193.84a	24.00a
	C + AMF + W	48.63ab	214.89a	21.33a	54.37a	252.49a	23.00a
	C + AMF + M + WW	43.20b	203.07a	21.67a	50.20a	201.35a	21.67a
2 WAT	C + W	88.93a	229.97a	32.00a	92.07a	233.18a	32.00a
	C + WS	103.37a	210.33a	40.00a	91.77a	261.96a	29.33a
	C + AMF + WS	100.90a	197.76a	35.00a	73.07a	198.52a	26.67a
	C + AMF + W	88.70a	147.69a	38.00a	90.57a	225.94a	30.67a
	C + AMF + M + WW	97.13a	192.69a	36.33a	72.70a	229.69a	25.33a
3 WAT	C + W	117.83a	219.32a	38.67ab	115.70a	247.99a	41.00b
	C + WS	103.37ab	166.45a	23.67c	83.03b	191.18a	21.67c
	C + AMF + WS	85.93b	176.49a	37.33b	75.27b	228.71a	42.00b
	C + AMF + W	116.03a	198.43a	49.33a	86.90ab	257.47a	51.67a
	C + AMF + M + WS	105.60a	198.44a	34.67bc	74.03b	243.59a	43.67b
4 WAT	C + W	118.73a	237.08a	37.33ab	120.53a	261.99a	45.67ab
	C + WS	102.40ab	144.62b	14.33c	83.37b	142.17b	9.00c
	C + AMF + WS	89.47b	171.95b	36.67ab	78.63b	200.48ab	36.67b
	C + AMF + W	118.23a	204.64ab	51.00a	90.27b	260.55a	55.00a
	C + AMF + M + WS	107.20ab	169.61b	27.00bc	75.17b	211.23ab	36.00b

Means with the same letter in the same column are not significantly different at $p < 0.05$ using DMRT.
Key: C- Cowpea, W- Watering, WS- Water stress, AMF- *Glomus deserticola,* M- *Macrophomina phaseolina,* WAT-week after treatment.

and numbers of leaves were highly significant at levels of treatment and days after treatment, but non-significant effect was recorded for leaf area. At replicate level, only plant height was highly significant, while leaf area and numbers of leaves per plant were not significant.

The drought tolerance of the cowpea cultivars were shown in Table 4. IT90K-277-2 shows significant difference ($p < 0.05$) in treatment at 7DAT with C + WD (1.00) and C + AMF + WD (1.00) not significantly different. 14DAT shows significant difference in treatment with C + AMF + M + WW and C + AMF + WD not significantly different, but significantly different from C + WD and C + AMF + WW which were not significantly different, while C + WW is significantly different from the treatments. These was also the observation recorded at 21DAT. At 28DAT C + WD, C + AMF + WW and C + AMF + M + WW were not significantly different with the value 2.00 for each treatment, but significantly different from C + WW (5.00) and C + AMF + WD (1.00) which are significantly different.

In IT84S-2246-4, at 7DAT significant difference was recorded in the treatment with C + WD (1.00) and C + AMF + WD (1.00), C + WW (1.67) and C + AMF + WW(1.67) were not significantly different ($p < 0.05$) respectively. Significant difference was also observed at 14DAT with C + WD (1.33) and C + AMF + M + WW (1.67) not significantly different. 21DAT shows non significance in treatments except C + WW (5.00) significantly different from the treatments. 28DAT recorded significant difference in the treatments except C + WD (1.33) and C + AMF + WD (1.00), C + AMF + WW (2.33) and C + AMF + M + WW (2.00) which shows non significance respectively.

The effect of treatment at different application of *Glomus. deserticola and Gigaspora. gigantea* on charcoal rot of cowpea is shown in Table 5. Cultivar IT90K-277-2 shows significant

Table 2
Effect of growth stages on before and after treatments on morphological characters of Cowpea cultivars T90K-277-2 and IT84S-2246-4.

	Before treatment				After treatment		
Days	Plant height(cm	Leaf area (cm^2)	No of Leaves	Days	Plant height(cm)	Leaf area (cm^2)	No of leaves
IT90K-277-2							
7 DBT	16.33^{d}	57.53^{c}	2.00^{d}	7 DAT	50.93^{c}	213.27^{a}	22.60^{b}
14 DBT	25.31^{c}	82.41^{b}	8.53^{c}	14 DAT	95.81^{b}	195.69^{a}	36.27^{a}
21 DBT	30.38^{b}	80.35^{b}	11.20^{b}	21 DAT	105.75^{a}	191.83^{a}	36.73^{a}
28 DBT	40.75^{a}	154.54^{a}	16.27^{a}	28 DAT	107.21^{a}	185.58^{a}	33.27^{a}
IT84S-2246-4							
7 DBT	14.20^{d}	48.26^{d}	2.00^{d}	7 DAT	52.88^{b}	221.69^{a}	22.67^{c}
14 DBT	21.07^{c}	69.53^{c}	7.93^{c}	14 DAT	83.49^{a}	228.68^{a}	29.36^{b}
21 DBT	27.15^{b}	96.90^{b}	10.33^{b}	21 DAT	87.46^{a}	236.91^{a}	41.36^{a}
28 DBT	39.27^{a}	203.07^{a}	16.40^{a}	28 DAT	90.19^{a}	221.20^{a}	38.57^{a}

Means with the same letter in the same column are not significantly different at $p < 0.05$ using Duncan Multiple Range Test (DMRT).
Key: DBT- Days before treatment, DAT- Days after treatment.

Table 3
Mean Square effect of treatment, replicates and stages of growth on growth characters of Cowpea cultivars.

		Before treatment					After treatment		
Source of variation	df	Plant Height	Leaf Area	No of Leaves	Source of variation	df	Plant height	Leaf area	No of leaves
IT90K-277-2									
corrected model	9				corrected model	9			
TRT	4	17.56^{ns}	733.88^{ns}	5.67^{ns}	TRT	4	378.50^{ns}	3764.32^{ns}	367.76**
REP	2	1.90^{ns}	1292.45^{ns}	3.15^{ns}	REP	2	262.64^{ns}	2094.44^{ns}	214.72^{ns}
DBT	3	1558.43**	26578.47**	529.31**	DAT	3	10523.28**	2114.65^{ns}	651.93**
Error	50	8.292	449.63	2.56	Error	50	167.11	1646.7	68.16
Total	60				Total	60			
Corrected Total	59				Corrected Total	59			
IT84S-2246-4									
corrected model	9				corrected model	9			
TRT	4	25.43**	1062.51*	3.92**	TRT	4	1271.26**	4084.32^{ns}	359.68**
REP	2	46.76**	324.94^{ns}	2.47^{ns}	REP	2	836.31**	981.6^{ns}	55.75^{ns}
WAS	3	1698.51**	70794.75**	532.82**	WAS	3	4194.61**	725.8^{ns}	995.18**
Error	50	3.63	404.79	1.19	Error	50	207.54	1790.24	66.94
Total	60				Total	60			
Corrected Total	59				Corrected Total	59			

*Are significantly different ($p < 0.05$) DMRT, (**) are significantly different ($p < 0.01$) DMRT, (^{ns}) are not significantly different ($p < 0.05$) DMRT.
Key: DAT-Days after treatment, DBT- Days before treatment, TRT-Treatment, REP- Replicate.

Table 4
Drought tolerance of two cowpea varieties as affected by *Glomus deserticola* and *Macrophomina phaseolina.*

		IT90K-277-2			IT84S-2246-4			
TREATMENT	7DAT	14DAT	21DAT	28DAT	7DAT	14DAT	21DAT	28DAT
C + W	1.00^{b}	2.00^{b}	2.00^{b}	2.00^{b}	1.00^{b}	1.33^{bc}	1.33^{b}	1.33^{c}
C + WS	1.67^{a}	5.00^{a}	5.00^{a}	5.00^{a}	1.67^{ab}	5.00^{a}	5.00^{a}	5.00^{a}
C + AMF + WS	2.00^{a}	2.33^{b}	2.00^{b}	2.00^{b}	1.67^{ab}	2.00^{b}	1.67^{b}	2.33^{b}
C + AMF + W	1.00^{b}	1.00^{c}	1.00^{c}	1.00^{c}	1.00^{b}	1.00^{c}	1.00^{b}	1.00^{c}
C + AMF + M + WS	2.00^{a}	1.00^{c}	1.33^{c}	2.00^{b}	2.00^{a}	1.67^{bc}	1.33^{b}	2.00^{b}

Means with the same letter in the same column are not significantly different at $p < 0.05$ using Duncan Multiple Range Test (DMRT).
Key: C- Cowpea, W- Watering, WS- Water stress, AMF- *Glomus deserticola,* M- *Macrophomina phaseolina,* DAT- Days after treatment.
Values are drought scale ratings of 1–5; 1(Excellent), 2(Good), 3(Moderate), 4(Poor), 5(Too poor).

Table 5
Effect of *Glomus deserticola* and *Gigaspora gigantea* on charcoal rot by *M. phaseolina.*

		IT90K-277-2			IT06K-123-1			
Treatment	Plant height (cm)	Leaf area (cm)	Stem girth (cm)	No. of leaves (cm)	Plant height (cm)	Leaf area (cm)	Stem girth (cm)	No. of leaves (cm)
Cowpea	40.88^{ef}	123.59^{a}	1.81^{cd}	16.67^{e}	37.20^{d}	164.79^{ab}	2.01^{a}	14.25^{fgh}
Cowpea + G. ds drench	44.12^{cde}	134.95^{a}	1.75^{cd}	17.50^{de}	39.98^{bcd}	153.75^{abc}	1.87^{ab}	16.08^{abcde}
Cowpea + G. ds simul.	35.12^{g}	93.04^{de}	1.69^{d}	13.67^{f}	38.96^{bcd}	157.06^{ab}	1.80^{bc}	16.33^{abcde}
Cowpea + G. gi drench	45.33^{bcd}	119.74^{ab}	1.7^{cd}	18.17^{de}	44.04^{a}	171.19^{a}	2.03^{a}	17.50^{a}
Cowpea + G.gi simul.	39.75^{f}	89.34^{e}	1.75^{cd}	20.42^{bc}	37.25^{d}	127.04^{cd}	1.90^{ab}	15.58^{cdef}
cowpea + M.phas.	31.13^{h}	62.55^{f}	1.52^{e}	9.50^{g}	30.05^{f}	99.70^{e}	1.57^{d}	10.58^{i}
cowpea + G.ds + M.phas + drench	44.06^{cde}	113.00^{bcde}	1.75^{cd}	19.25^{cd}	37.62^{cd}	146.40^{abcd}	1.79^{bc}	13.58^{gh}
cowpea + G.ds + M.phas + simul.	40.88^{ef}	95.27^{cde}	1.73^{cd}	16.50^{e}	34.50^{e}	121.71^{de}	1.71^{c}	13.00^{h}
Cowpea + G. gi + M.phas drench	49.48^{a}	113.47^{bcd}	1.93^{b}	23.83^{a}	39.15^{bcd}	125.06^{d}	1.83^{bc}	15.00^{efg}
Cowpea + G.gi + M.phas + simul.	42.74^{def}	122.49^{ab}	1.81^{cd}	19.33^{cd}	40.32^{bc}	138.29^{bcd}	1.90^{ab}	15.67^{bcdef}
Cowpea + G.ds + G. gi + M.phas drench	44.12^{cde}	117.52^{bc}	1.83^{bc}	20.33^{bc}	40.94^{b}	143.05^{abcd}	1.87^{ab}	15.17^{def}
Cowpea + G.ds + G.gi + M. phas + simul.	42.71^{def}	111.9^{bcde}	1.77^{cd}	17.42^{de}	39.71^{bcd}	158.86^{ab}	1.83^{bc}	16.75^{abcd}
Cowpea + G.ds + G. gi drench	49.11^{ab}	143.61^{a}	2.10^{a}	22.25^{ab}	41.76^{ab}	170.06^{a}	1.88^{ab}	17.25^{ab}
Cowpea + G.ds + G.gi simul.	48.08^{bc}	119.7^{ab}	2.07^{a}	20.67^{bc}	41.08^{b}	155.62^{ab}	1.93^{ab}	16.83^{abc}

Means with the same letter in the same column are not significantly different at $p < 0.05$ using Duncan Multiple Range Test (DMRT).
Key: G. ds- *Glomus deserticola, G. gi- Gigaspora gigantean, M. phas- Macrophomina phaseolina,* simul.- simultaneous.

differences ($p < 0.05$) within the treatment for the characters. Application of *G. deserticola* and *G. gigantea* were effective in ameliorating the negative effect of *M. phaseolina,* while soil treated with *G. gigantea* before planting (drench) + *M. phaseolina* recorded the highest value in plant height and numbers of leaves with 49.48 cm and 23.83 respectively. It is observed in the plant height that treatment with Cowpea + *G.deserticola* drench, cowpea + *G. deserticola*+ *M.phaseolina* + drench and Cowpea + *G.deserticola* + *G.*

gigantean + *M.phaseolina* drench were not significantly different ($p < 0.05$). The control treatments combination of *G. deserticola* + *G. gigantean* drench (143.61 cm^2) and cowpea alone (123.59 cm^2) recorded the highest value of leaf area with cowpea alone (123.59 cm^2), cowpea + *G. deserticola* drench (134.95 cm^2) and cowpea + *G. deserticola* + *G. gigaspora* drench (143.61 cm^2) not significantly different. Significant difference was recorded on the stem girth with cowpea + *G. deserticola* + *G. gigaspora* drench and cowpea + *G. deserticola* + *G. gigaspora* recording the highest value of 2.10 and 2.07 cm respectively. Also, treatment with the pathogen alone (*Macrophomina phaseolina*) recorded least value in all the growth parameters; plant height (31.13 cm), leave area (62.55 cm^2), stem girth (1.52 cm) and numbers of leaves (9.50). Result also show that of all treatments *G. gigantea* and the combination of *G. deserticola* + *G. gigantea* were the most effective in the cultivar.

The cultivar, IT06K-123-1 shows significant difference $p < 0.05$ between the control and other treatments in all the growth characters. For plant height result shows that cowpea + *G. deserticola* drench (39.98 cm), cowpea + *G. deserticola* simul (38.96 cm)., cowpea + *G. gigantean* + *M. phaseolina* drench (39.15) and cowpea + *G. deserticola* + *G. gigaspora* + *M. phaseolina* simul. (39.71 cm) were not significantly different ($p < 0.05$). There was significant difference in leaf area with cowpea + *G. deserticola* + *M. phaseolina* drench (146.40 cm^2) and cowpea + *G. deserticola* + *G. gigaspora* + *M. phaseolina* drench (143.05 cm^2) not significantly different. Treatments recorded significant difference for stem girth with cowpea alone and cowpea + *G. gigaspora* drench recording the highest value of 2.01 and 2.03 cm respectively. There was variation in numbers of leaves, showing significant difference in treatment except cowpea + *G. deserticola* drench (16.08) and cowpea + *G. deserticola* simul. (16.33) which were not significantly different. However, the control cowpea + *G. gigantea* (drench) recorded the highest value in all growth parameters followed in the trend is *G. deserticola* + *G. gigantea* and cowpea alone. While treatment with cowpea + *M. phaseolina* recorded the least value in growth characters.

Table 6 shows that disease severity was significantly different ($p < 0.05$) between the treatments and control which shows no incidence of disease. In cultivar IT90K-277-2 treatment with *M. phaseolina* was significantly different from treatment not inoculated. Treatment with cowpea + *G. deserticola* + *M. phaseolina simul.* (7.92), cowpea + *G. gigantea* + *M. phaseolina* simul. (7.92), cowpea + *G. deserticola* + *G. gigantea* drench and simultaneous (5.83) were not significantly different. Treatment with the combination *G. deserticola* + *G. gigantea* (drench and simultaneous) was most effective recording resistance. While, treatment with cowpea + *G. gigantea* (drench) and cowpea + *G. deserticola* (drench) were the least effective in the cultivar and Cowpea + *M. phaseolina* recorded high susceptibility. Cultivar IT06K-123-1 shows high susceptibility to the pathogen while treatments with AMF werw resistant.

4. Discussion

Watanabe et al. [3] reported that there are variation tolerating ability of some cowpea genotypes to drought condition in the vegetative state. On the other hand, Turk et al. [5] cited in Ahmed and Suliaman [6] also affirmed that cowpea is highly sensitive to water stress during the flowering and pod-filling stages. These were in accordance with the observations in this study as the two cowpea cultivars (IT90K-277-7 and IT84S-2446-4) responded in variance to drought when inoculated with *Glomus deserticola* at vegetative stage. IT84S-2446-4 had higher tolerance to drought compared to IT90K-277-7. The performance of *G. deserticola* treated plants also agreed with the findings of Ruiz-Lanzo et al. [33], and Olawuyi et al. Olawuyi et al. (2014a,b). The increase in plant height due to period of inoculation, production of numbers of leaves and leaf area of cowpea cultivars inoculated with *G. deserticola* were similarly observed by Quilambo [34] and Olawuyi et al. [26]. Prolong water stress observed from the study could be due to the reduction of *G. deserticola* effectiveness as reported by Ryan and Ash [35] and Bryla and Duniway [36], as mycorrhiza fungi may alleviate moderate drought stress but becomes ineffective in severe drought condition.

The reduction in number of pod per plant and seed per plant were observed in treatment with water stress compared to the control. This supported the observation reported by some other researchers [37]. Although, Ravindra et al. [38], attributed the loss in seed yield to low fruiting efficiency and lack of filling time for pods, while Turk and Hall [39] linked the reduction in seed yield under water stress to the secondary detrimental effect of drought avoidance on CO_2 assimilation. The significant reduction in number of harvested pods per plant under water stress could also be attributed to abscission of the reproductive structure [40].

The plant treated with *G. deserticola* exhibited an improved drought tolerance compared to the non mycorrhizal treatment under stress. This confirmed the report of Smith and Read (1997) who stated the ability of Vesicular Arbuscular Mycorrhiza (VAM) fungi to substantially increase the host plant's tolerance to water stress. Auge [29] reported that the mechanism used by VAM increased the root hydraulic conductivity, improved stomatal regulation, osmotic adjustment in host and enabled extraction of water from smaller pores through improved contact with soil particles as a result of the hyphae binding effect.

Table 6
Severity index and susceptibility class of cowpea cultivars IT90K-277-2 and IT06K-123-1 to charcoal rot disease.

Treatment	Disease severity index		Plant rating		Susceptibility/resistance class	
	IT90K-277-2	IT06K-123-1	IT90K-277-2	IT06K-123-1	IT90K-277-2	IT06K-123-1
Cowpea	0.00^{d}	0.00^{c}	0	0	Highly resistant	Highly resistant
Cowpea + G. ds drench	0.00^{d}	0.00^{c}	0	0	Highly resistant	Highly resistant
Cowpea + G. ds simul.	0.00^{d}	0.00^{c}	0	0	Highly resistant	Highly resistant
Cowpea + G. gi drench	0.00^{d}	0.00^{c}	0	0	Highly resistant	Highly resistant
Cowpea + G.gi simul.	0.00^{d}	0.00^{c}	0	0	Highly resistant	Highly resistant
cowpea + M.phas.	42.08^{a}	58.30^{a}	5	4	Highly susceptible	Highly susceptible
cowpea + G.ds + M.phas + drench	12.92^{b}	7.50^{b}	3	2	resistant	resistant
cowpea + G.ds + M.phas + simul.	7.92^{c}	8.75^{b}	2	2	resistant	resistant
Cowpea + G. gi + M.phas drench	12.5^{b}	10.00^{b}	3	2	resistant	resistant
Cowpea + G.gi + M.phas + simul.	7.92^{c}	9.58^{b}	2	2	resistant	resistant
Cowpea + G.ds + G. gi + M.phas drench	5.83^{c}	5.00^{bc}	2	1	resistant	Highly resistant
Cowpea + G.ds + G.gi + M.phas + simul.	5.83^{c}	5.00^{bc}	2	1	resistant	Highly resistant
Cowpea + G.ds + G. gi drench	0.00^{d}	0.00^{c}	0	0	Highly resistant	Highly resistant
Cowpea + G.ds + G.gi simul.	0.00^{d}	0.00^{c}	0	0	Highly resistant	Highly resistant

There was interaction of VAM fungi with plant pathogenic organism. The establishment of VAM in the root of host plant, primarily reduced diseases caused by soil borne pathogen [41]. Previous findings had also revealed that mycorrhizal plant offer increased tolerance to fungal root pathogen [22]. In this study the effect of the interaction between *G. deserticola*, *G. gigantea* and *M. phaseolina* investigated, withstand the effect of pathogen which could have resulted in great reduction in plant height and numbers of leaves which might likely reduce the photosynthetic ability of the plant thus, resulting into yield loss. Therefore plants treated with both pathogen and AMF attained normal growth. Dar et al. [42], had earlier reported that enhance plant growth improved nutrient assimilation and physical barrier that offered resistance to the plants.

The observation from this study shows soil drenched with AMF before planting improved cowpea plant compared to the un-inoculated soil before planting. This could be as a result of the potential of AMF in making the immobile phosphorous readily available in the soil. Several investigations indicate that there is a beneficial effect of VA- fungi inoculation on nutrient uptake and plant growth especially in sterilized soils [43,44]. The combination of AMF (*G. deserticola* and *G.gigantea*) were the most effective in improving the tolerance traits and disease resistance of the cowpea cultivars.

The *M. phaseolina* and AM fungi exploited common resources which included infection site, space and photosynthate within the root [45]. Interference competition may also arise if there is carbon availability within intercellular spaces and rhizophere [46]. The number of infection loci within the root system was reduced as a result of AM fungal colonization

[47]. The two cowpea cultivars showed high susceptibility to *M. phaseolina*, but, AMF inoculated plant offered resistance to the pathogen. The potentials of AMF in protecting plants from root pathogens which included; *Phytophthora parasitica* or *Fusarium* sp., root- invading nematodes had been previously reported [48,49,22,50,23].

5. Conclusion

It is evident from this study that IT 84S-2446-4 had better tolerance to drought compared to IT90K-277-7 when inoculated with *Glomus deserticola*, the treatment combination of Arbuscular mycorrhiza fungi (*Glomus deserticola* and *Gigaspora gigantea*) had better potentials to tolerate the effect on susceptible Cowpea cultivars(IT90K-277-7 and IT84S-2446-4) to drought as well as resisting the pathogen (*Macrophomina phaseolina*) causing charcoal rot disease of cowpea.

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