

Arbuscular Mycorrhizal Fungi and *Trichoderma* spp Influence on Nutrient Uptake and Water Stress Tolerance in Cowpea (*Vigna unguiculata* L. Walp)

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Abstract

Drought and nutrient un-availability are amongst the major environmental stresses that hinder plants productivity. The use of synthetic fertilizers in drought-stricken lands is unaffordable to most small-holder farmers and contributes to environmental pollution. This study was conducted to determine the influence of Arbuscular Mycorrhizal fungi and *Trichoderma* spp on nutrient uptake in cowpea at different soil water levels under greenhouse conditions. The experiment was conducted using a $3 \times 2 \times 8$ factorial arranged on a completely randomized block design with the three water levels treatment of 90 % (no stress), 60 % (mild stress), and 30 % (severe stress) of field capacity (FC). Two cowpea varieties (KVU 27-1 and K80) were used in each water level and each was subjected to eight fungal inoculation treatments: *Funneliformis mosseae*, *Rhizophagus irregularis*, *Trichoderma harzianum*, *Trichoderma asperellum*, *Funneliformis mosseae* + *Rhizophagus irregularis*, *Trichoderma harzianum* + *Trichoderma asperellum*, *T. harzianum* + *Funneliformis mosseae* + *Rhizophagus irregularis*, and control (un-inoculated) that were in the replicates of four. The shoot tissue analysis was done to determine the nitrogen (N), phosphorous (P) and potassium (K) contents. The data were subjected to a Two-Way ANOVA to determine the influence of the two fungi on nutrient uptake. Means were separated using Bonferroni at $p < 0.05$. Results showed that all the shoot nutrient contents were significant at ($p < 0.05$) with soil water level treatment in both varieties. Water stress negatively influenced shoot nutrient contents. The combined inoculation of HarS and HBB greatly influenced shoot nutrient levels for both cowpea varieties than the controls. The highest N and P shoot content were 3.7 % (in KVU 27-1) and 0.12 % (in K80) inoculated with HBB and HarS respectively. The highest K content was 2.7 % in both varieties inoculated with HarS. Therefore, co-inoculation of HarS and HBB was the most appropriate to nutrient uptake in the cowpea varieties.

Key words: drought, nutrients, inoculation, productivity, field capacity

Introduction

Amongst the physiological stresses that limit crop growth are drought and nutrient availability. These stressors culminate in poor agricultural yields, a characteristic of arid and semi-arid lands (Abobatta, 2019). Drought also hampers root development (Chun *et al*, 2021) which adversely affects water and nutrient uptake. The use of synthetic

fertilizers to amend soil nutrition status in drought-stricken lands is not economically sustainable for most smallholder farmers and has also been a major contributor to environmental pollution (Oruru *et al.*, 2018). Arbuscular mycorrhizal fungi and *Trichoderma* spp have been proposed as an alternative to promote growth by enhancing nutrient and water uptake (Stewart & Hill,

2014). Arbuscular mycorrhizal fungi (AMF) are purely endomycorrhizal as they exhibit invasive symbiosis (Tamasloukht *et al.*, 2003) and facilitates uptake of essential nutrients by the host plants leading to faster growth for interchange of plant carbon (Hashem *et al.*, 2018). *Trichoderma* spp are opportunistic endophytes that live in soil and roots influencing plant growth and resistance against pathogens (Samuels, 2006).

Through their mutual association with plants' roots, AMF's widely spread hyphal web increases acquisition of essential nutrients such as phosphorous from soil (Arihara & Karasawa, 2012). Oruru *et al.* (2018) recorded higher amounts of shoot nitrogen and phosphorous in mycorrhizal inoculated cowpea than in the un-inoculated ones. The increased nutrients levels in plants promotes enzymatic activities leading to early establishment of plants thereby escaping harsh environmental stresses like drought. The AMF protect plants against drought injury by enhancing root development and conductivity that promote higher nutrient and water status in plants (Begum *et al.*, 2019). These beneficial fungi also sustain stomatal conductance during drought stress through regulation of abscisic acid (ABA) synthesis (Diagne *et al.*, 2020). Additionally, AMF promotes the accumulation of compatible osmolytes including jasmonic acid that reinforces osmotic adjustment to enable plants maintain turgor and physiological activity (Ntombela, 2012).

Trichoderma spp on the other hand has been used to control phytopathogens which sustains plant immunity and promotes physiological activity and growth (Harman *et al.*, 2004). *Trichoderma* spp also promote growth and development of the root system which facilitates improved water and nutrient uptake in the event of water deficit (Azarmi *et al.*, 2011). This results in increased photosynthesis

and reduced oxidative stress under water stress (Khoshmanzar *et al.*, 2019). Inoculation of plants with *Trichoderma* spp also initiates production of hormones and secondary metabolites which have various promotory effects on plants. Chagas *et al.*, (2016) recorded the production of IAA after inoculating cowpea with different isolates of *Trichoderma* which improved biomass. Secondary metabolites promote growth through early germination and early plant establishment (Stewart & Hill, 2014).

A mixture of *Trichoderma* isolates is capable of enhancing greater bioactivity than when one isolate is used. The inoculation of both *T. harzianum* and *T. asperellum* was successfully used to inhibit growth of *Botrytis cinerea* in tomato (Kuzmanovska *et al.*, 2018). Dual inoculation of AMF and *Trichoderma* spp stimulate synergistic effects that lead to increased growth and drought tolerance. (Metwally and Al-Amri, 2019) recorded increased biomass and bulb diameter in onions after it was co-inoculated with *Trichoderma viride* and AMF consortium.

Cowpea (*Vigna unguiculata* (L). Walp), is an essential leguminous crop in Kenya and also globally. The plant serves as food for humans, livestock and is also sold for income mostly in marginal areas (ASALs) (Owade *et al.*, 2020). Additionally, cowpea improves soil fertility by fixing nitrogen in the soil (Guimarães *et al.*, 2012) and is drought tolerant. In spite of its advantages, cowpea has recorded a low output in Africa due to droughts and infertility (Ojiewo *et al.*, 2019). Cowpea, tolerates low soil fertility due to its nitrogen fixing ability. Extreme droughts, however, lower absorption of other nutrients which interferes with rhizobial activity at the cowpea roots (Oruru *et al.*, 2018). The pursuit for greater agricultural production has led to excessive utilization of chemical fertilizers to increase crop yields. Use of chemical fertilizers

increases risks of soil salinization, causes environmental pollution and it is not cost effective for smallholder farmers (Oruru et al., 2018). The utilization of growth-promoting microorganisms such as AMF and *Trichoderma* spp has been underscored in sustaining high yields under adverse environmental stresses. Even though these microorganisms are naturally found in most agricultural and desert soils, their precise effect on promotion of nutrient uptake on cowpea has not been clearly addressed. Additionally, soil water management plays a crucial role in optimizing water usage for crop production. There's however no documentation of precise watering requirement for cowpea. This study hypothesized that AMF and *Trichoderma* spp have no effect on nutrient uptake in cowpea under different water levels. This study was conducted to assess the influence of AMF and *Trichoderma* spp on nutrient uptake in cowpea under different soil water levels. Results from the research serve to inform use of these inoculants as biofertilizers and improvement production of cowpea and other crops.

Materials and Methods

Study area

The study was carried out in the tissue culture laboratory of the Department of Plant Sciences of Kenyatta University, Kenya. Monitoring of cowpea growth was done in a greenhouse at the department while the laboratory was used for culturing and nutrient analysis.

Experimental design and treatments

The green house experiment was conducted using a $3 \times 2 \times 8$ factorial arranged in a complete randomized block design (CRBD). The three water levels were at 90 % (no stress), 60 % (mild stress), and 30 % (severe stress) of field capacity (FC). Two cowpea varieties (KVU 27-1 and K80) were each treated with eight fungal inoculation treatments: I- *Funneliformis mosseae* (BEG

12), II- *Rhizophagus irregularis* (BEG 44), III- *Trichoderma harzianum* (Har), IV- *Trichoderma asperellum* (Asp), V- BEG 12 + BEG 44 (BB), VI- Har + Asp (HarS), VII- Har + BEG 12 + BEG 44 (HBB), and VIII- Control (un-inoculated). Each experimental unit had four replicates resulting in 192 pots. The pots measured 17 cm diameter and 16 cm height and the substrate used was sterile forest soil and sand.

Soil collection and analysis

Forest soil was used as a substrate and was mixed thoroughly with sand in the ratio 3:1 respectively. The mixed substrate was passed through a 2 mm sieve and then autoclaved at 121 °C, 1.5 psi for 1 hour. A sample of the soil was analyzed at the soil testing laboratory of Kenya Agricultural and Livestock Research Organization (KALRO), Nairobi for physiochemical properties using procedures by Okalebo et al. (2002) as presented in table 1

Table 1: Soil survey analysis

Soil properties	Value
Soil pH	5.22
Exchange acidity (meq %)	0.3
Total nitrogen %	0.8
Total Organic Carbon %	8.32
Phosphorous (ppm)	15
Potassium (meq %)	0.64
Calcium (meq %)	3.20
Magnesium (meq %)	1.41
Manganese (meq %)	0.79
Copper (ppm)	1.00
Iron (ppm)	59.4
Zinc (ppm)	3.60
Sodium (meq %)	0.28

Planting material

Seeds of two cowpea cultivars (KVU 27-1 and K80) were purchased from Kenya Agricultural Livestock and Research Organization (KALRO). They were surface sterilized by washing them in 70 % ethanol which was followed by addition of 2 % NaOCl for a

duration of two minutes before they were rinsed severally with sterile water.

The fungal inocula

The AMF consisted of indigenous *Funneliforms mosseae* and *Rhizophagus irregularis* that had been colonized with Bermuda grass (*Cynodon dactylon*) roots and spores to provide 10 spores/g of each AMF. *Trichoderma spp* consisted of two isolates namely *T. harzianum* and *T. asperellum* obtained from the repository of Kenyatta University in the Biochemistry, Microbiology and Biotechnology Department. The isolates were rejuvenated by growing them separately on fresh PDA media. The culture was converted to a suspension culture of 10^6 spores/ml (Halifu *et al.*, 2019) which was used to inoculate the seeds.

Experiments

For inoculation with AMF, a concentration of 10 spores/g AMF of the ground roots of Bermuda grass was used. Five grams of AMF inoculant were placed 3 cm in holes made in the pot and mixed thoroughly with soil prior to sowing. Where dual inoculation of the two AMF (*F. mosseae* and *R. irregularis*) was involved, 2.5 g of each species were mixed thoroughly with soil in a hole. For *Trichoderma*, seed lots were treated by placing them in a *Trichoderma* spore suspension of concentration 10^6 spores/ml followed by addition of a small quantity of Gum Arabica to reinforce the attachment of fungi on the seeds. This was left for 24 hours after which the seeds were air-dried before sowing. Each pot was sown with five cowpea seeds each seed at an approximate depth of 3 cm, and a spacing of 6 cm between holes. Tap water was put in the pots immediately and water content maintained at 90 % FC for 14 days to allow for seedling establishment. Seedlings were thinned to retain two cowpea plants per pot. Pots were subjected to respective water levels;

60 % and 30 % FC. HydroSense II (HS2 and HS2P, Campbell Scientific, Inc) was used to measure the soil water capacity daily in the morning until the 40th day. Whenever the soil water content was below the required water levels, irrigation to the three water levels was done. Forty days after planting harvesting was done, shoots separated from roots and shoots were oven-dried at 72 °C to obtain dry samples.

Data collection

The dry shoot samples were ground to a homogenous composite and ashed at 400 °C for 1 hour. Five grams of each sample were chemically digested using a mixture of salicylic acid, H₂SO₄, H₂O₂ and selenium (Novozamsky *et al.*, 1983). Potassium content was established through Flame Photometry, phosphorus was calorimetrically determined on a spectrophotometer, while Nitrogen was determined calorimetrically using Segmented Flow Analyser (Walinga *et al.*, 1989).

Data analysis

All data were analyzed for variance via Two-Way analysis of variance (ANOVA) to determine the influence of AMF and *Trichoderma spp* on the nutrient uptake at different water levels in cowpea. The factors were fungal inoculum, soil water level and cowpea varieties, though data of cowpea varieties were analyzed separately. Where variations of means were significant at $\alpha=0.05$, multiple mean comparison was done using Bonferroni. Statistical analyses were performed using R (software version 4.2.1).

Results

Results showed that there was a significant increase ($p < 0.05$) in N, P, and K contents in the shoots of both KVVU 27-1 and K80 cowpea varieties (Figures 1, 2, and 3). The shoot nutrient content increased significantly with increase in water level from 30 to 90 %.

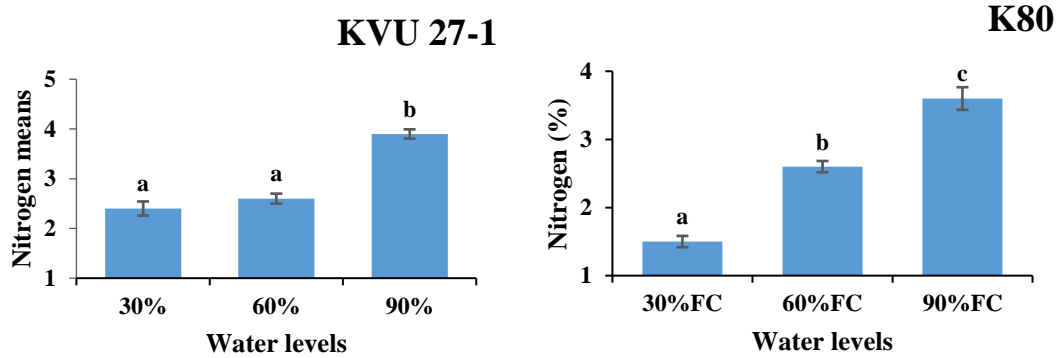


Figure 1: Nitrogen content in the two cowpea varieties subjected to 30%, 60% and 90% soil water levels. Bars with different letters indicate that the means are significantly different.

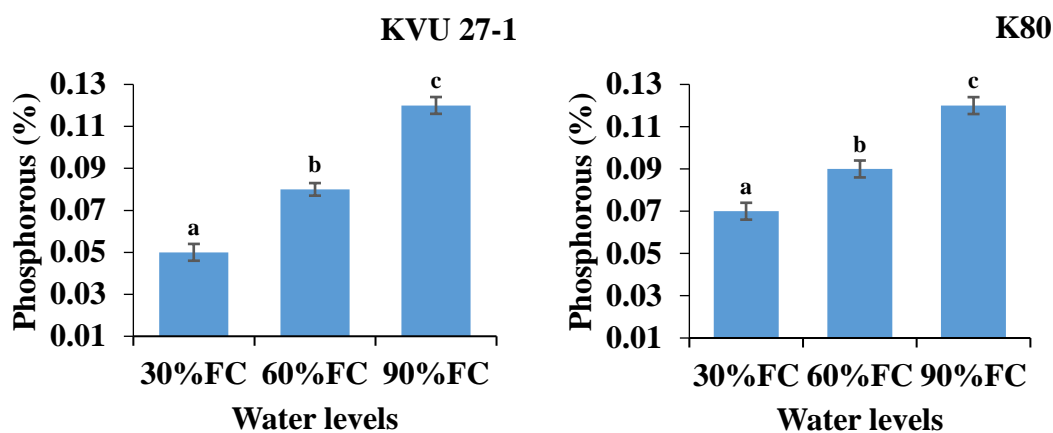
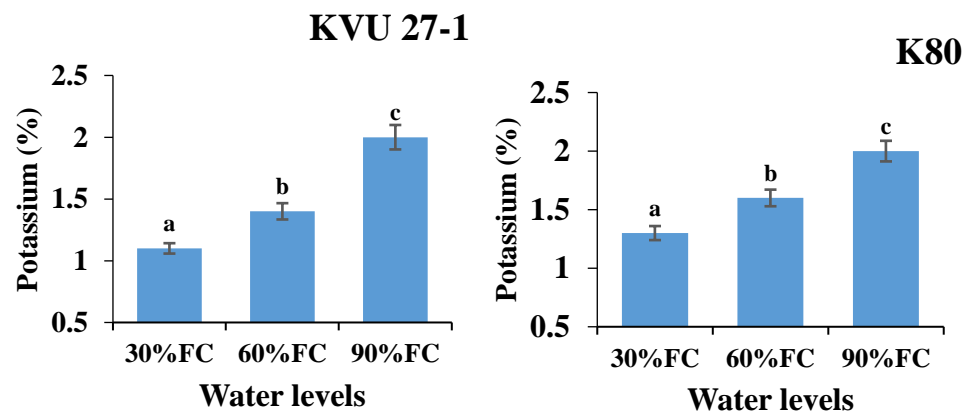


Figure 2: Phosphorous content in the two cowpea varieties subjected to 30%, 60% and 90% soil water levels. Bars with different letters indicate that the means are significantly different.



Figures 3: Potassium content in the two cowpea varieties subjected to 30%, 60% and 90% soil water levels. Bars with different letters indicate that the means are significantly different.

The fungal inoculants had a significant effect ($p < 0.05$) on N, P and K contents in both cowpea varieties. The combined inoculations

of HBB in KVVU 27-1 and HarS in K80 had the highest nitrogen content of 4.46 % and 5.56 % respectively both at 90 % FC (Figure 4).

Likewise, the shoot phosphorous contents were highest of 0.14 % and 0.15 % in KVV 27-1 and K80 with the combined inoculation of HBB and HarS respectively (Figure 5). For potassium, the co-inoculation of HarS caused the highest values of 3.25 % and 2.69 % for both cowpea varieties (Figure 6). The

interaction between water levels and the fungal inocula was significant ($p < 0.05$) for N and K in both varieties (Tables 2 and 3). For P, the interaction of these two factors was significant only in K80 but was insignificant in KVV 27-1 (Table 4).

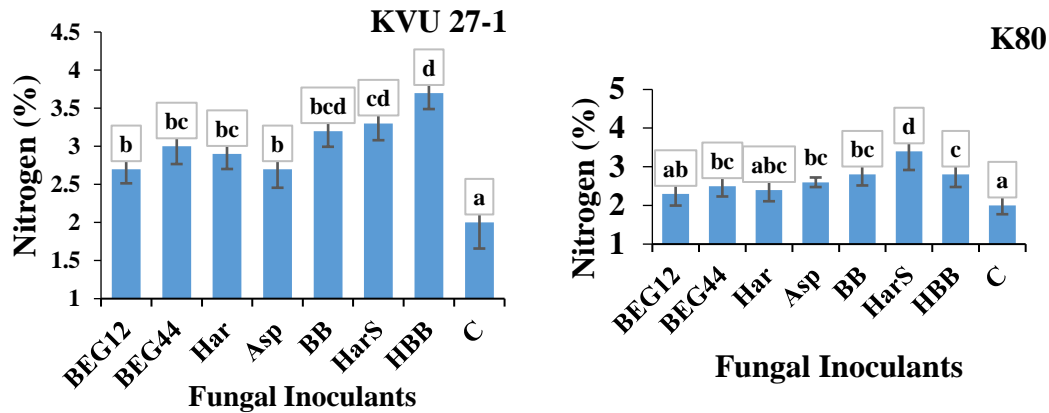


Figure 4: Means of Nitrogen content at different fungal inocula. Bars with different letters within indicate that the means are significantly different. BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control (un-inoculated).

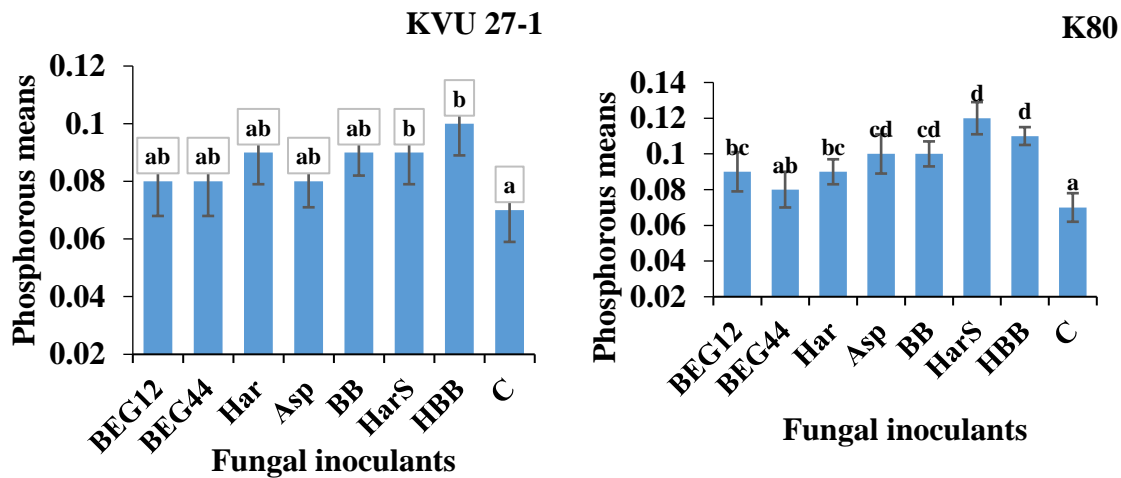


Figure 5: Means of phosphorous content at different fungal inocula. Bars with different letters within indicate that the means are significantly different. BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control (un-inoculated).

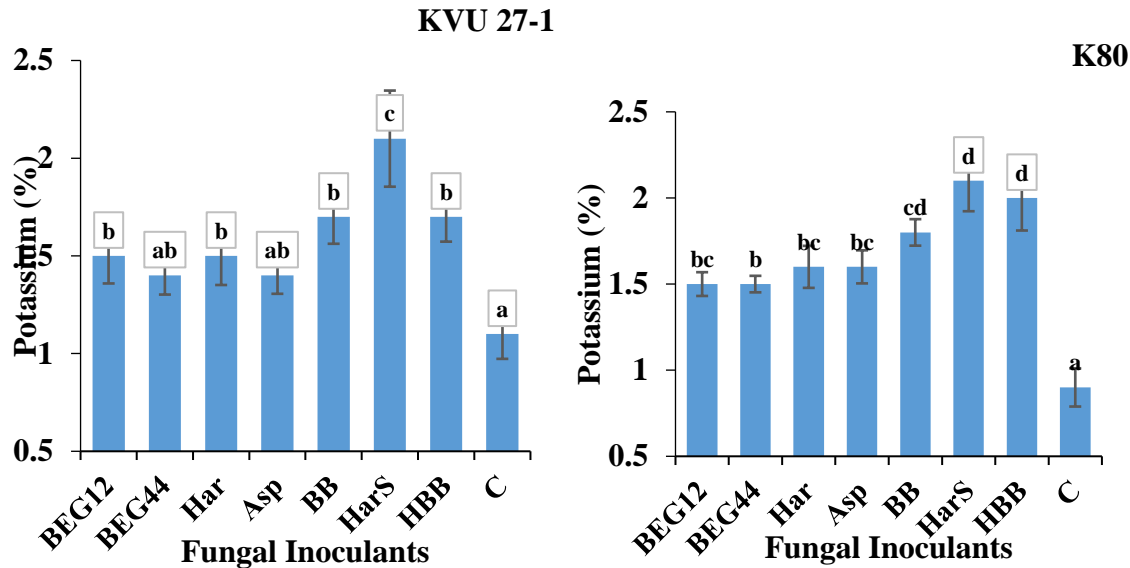


Figure 6: Means of potassium content at different fungal inocula. Bars with different letters within indicate that the means are significantly different. BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control (un-inoculated).

Table 2: Effect of AMF and *Trichoderma* spp on nitrogen content of cowpea at three soil water levels

Cowpea Varieties	KVVU 27-1			K80		
	Soil water level			Soil water level		
Fungal inoculant	30%FC	60%FC	90% C	30%FC	60%FC	90% C
BEG 12	2.27±0.21 ^{bcd}	2.38±0.07 ^{bcde}	3.51±0.14 ^{efghij}	1.00 ±0.76 ^a	2.65 ±0.20 ^{efghij}	3.30 ±0.14 ^{ghijk}
BEG44	2.25±0.29 ^{bcd}	3.01±0.15 ^{cdefgh}	3.88±0.22 ^{hij}	1.46 ±0.10 ^{abc}	2.52 ±0.27 ^{efghi}	3.46 ±0.19 ^{ijk}
Har	2.40±0.17 ^{bcde}	2.65±0.17 ^{bcdefg}	3.64±0.30 ^{fghij}	1.34 ±0.09 ^{ab}	2.34 ±0.23 ^{cdefg}	3.57 ±0.19 ^{jk}
Asp	2.00±0.13 ^{abc}	2.39±0.14 ^{bcde}	3.77±0.21 ^{ghij}	2.04 ±0.11 ^{bcdef}	2.77 ±0.08 ^{efghij}	2.91 ±0.07 ^{fghij}
BB	3.21±0.22 ^{defghi}	2.60±0.25 ^{bcdef}	3.91±0.26 ^{hij}	1.51 ±0.18 ^{abcd}	3.38 ±0.16 ^{hijk}	3.43 ±0.15 ^{hijk}
HarS	2.71±0.23 ^{cdefg}	2.94±0.17 ^{cdefgh}	4.22±0.09 ^{ij}	2.21 ±0.14 ^{bcdef}	2.46 ±0.08 ^{defgh}	5.56 ±0.46 ^l
HBB	3.41±0.26 ^{efghi}	3.18±0.11 ^{defghi}	4.56±0.22 ^j	1.84 ±0.07 ^{abcde}	2.30 ±0.11 ^{bcdef}	4.21 ±0.28 ^k
Control	0.98±0.08 ^a	1.52±0.15 ^{ab}	3.50±0.29 ^{efghij}	1.02 ±0.06 ^a	2.18 ±0.12 ^{bcd}	2.75 ±0.17 ^{efghij}
P values						
Fungal inoculant	0.000			0.000		
Water level	0.000			0.000		
Fungal inoculant*Water level	0.001			0.000		

Table 3: Effect of AMF and *Trichoderma* spp on the phosphorous content of cowpea at three soil water levels

Cowpea Varieties	KVU 27-1			K80		
	Soil water level			Soil water level		
Fungal inoculant	30%FC	60%FC	90% C	30%FC	60%FC	90% C
BEG 12	0.04±0.02 ^{ab}	0.08±0.01 ^{abcdefgh}	0.12±0.00 ^{fgh}	0.05 ±0.01 ^{ab}	0.10 ±0.00 ^{cdefg}	0.13 ±0.01 ^{ghi}
BEG44	0.04±0.01 ^a	0.08±0.00 ^{abcdefgh}	0.13±0.01 ^{fgh}	0.04±0.00 ^a	0.08 ±0.01 ^{abcde}	0.12 ±0.00 ^{fghi}
Har	0.05±0.01 ^{abc}	0.08±0.01 ^{abcdefgh}	0.13±0.00 ^{gh}	0.07 ±0.01 ^{abcd}	0.10 ±0.01 ^{cdefg}	0.12 ±0.00 ^{fghi}
Asp	0.06±0.01 ^{abcde}	0.07±0.01 ^{abcdef}	0.12±0.01 ^{defgh}	0.06 ±0.01 ^{abc}	0.10 ±0.00 ^{defgh}	0.14 ±0.00 ^{hi}
BB	0.06±0.01 ^{abcde}	0.08±0.00 ^{abcdefgh}	0.12±0.01 ^{efgh}	0.09 ±0.01 ^{bcdef}	0.10 ±0.02 ^{defgh}	0.12 ±0.00 ^{fghi}
HarS	0.07±0.01 ^{abcdef}	0.08±0.01 ^{abcdefg}	0.14±0.01 ^h	0.09 ±0.00 ^{bcdefg}	0.11 ±0.00 ^{defgh}	0.15 ±0.01 ⁱ
HBB	0.06±0.01 ^{abcde}	0.10±0.00 ^{bcdefgh}	0.14±0.02 ^h	0.11 ±0.00 ^{defgh}	0.12 ±0.01 ^{efghi}	0.12 ±0.01 ^{fghi}
Control	0.04±0.01 ^{ab}	0.06±0.01 ^{abcd}	0.10±0.02 ^{cdefgh}	0.04 ±0.00 ^a	0.07±0.01 ^{abcd}	0.10 ±0.00 ^{defg}
P values						
Fungal inoculant	0.020			0.000		
Water level	0.000			0.000		
Fungal inoculant*Water level	0.721			0.002		

Table 4: Effect of AMF and *Trichoderma* spp on the potassium content of cowpea at three soil water levels

Cowpea Varieties	KVU 27-1			K80		
	Soil water level			Soil water level		
Fungal inoculant	30%FC	60%FC	90% C	30%FC	60%FC	90% C
BEG 12	1.11±0.04 ^{abcde}	1.22±0.06 ^{abcdef}	2.06±0.18 ^{ghi}	1.27 ±0.08 ^{bc}	1.63 ±0.08 ^{cdef}	1.72 ±0.04 ^{cdef}
BEG44	1.06±0.06 ^{abcd}	1.37±0.12 ^{abcdefg}	1.67±0.16 ^{defghi}	1.40 ±0.09 ^{bcde}	1.53 ±0.05 ^{bcdef}	1.61 ±0.09 ^{cdef}
Har	0.97±0.04 ^{abc}	1.66±0.22 ^{cdefghi}	1.78±0.26 ^{efghi}	1.33 ±0.08 ^{bcd}	1.46 ±0.05 ^{bcde}	2.16 ±0.15 ^{fg}
Asp	1.11±0.10 ^{abcde}	1.33±0.11 ^{abcdef}	1.66±0.16 ^{cdefghi}	1.34 ±0.05 ^{bcd}	1.61 ±0.05 ^{cdef}	1.96 ±0.18 ^{def}
BB	1.23±0.13 ^{abcdef}	1.59±0.06 ^{bcdefghi}	2.25±0.09 ⁱ	1.55 ±0.06 ^{bcdef}	1.93 ±0.09 ^{cdef}	2.04 ±0.11 ^{efg}
HarS	1.22±0.10 ^{abcdef}	1.85±0.08 ^{fghi}	3.15±0.07 ^j	1.61 ±0.08 ^{cdef}	1.88 ±0.34 ^{cdef}	2.69 ±0.04 ^g
HBB	1.40±0.18 ^{abcdefg}	1.45±0.07 ^{abcdefg}	2.18±0.10 ^{hi}	1.52 ±0.06 ^{bcdef}	1.79 ±0.17 ^{cdef}	2.68 ±0.03 ^g
Control	0.91±0.10 ^a	0.77±0.12 ^{ab}	1.55±0.20 ^{bcdefgh}	0.56 ±0.08 ^a	0.93 ±0.15 ^{ab}	1.32 ±0.10 ^{bcd}
P values						
Fungal inoculant	0.000			0.000		
Water level	0.000			0.000		
Fungal inoculant*Water level	0.000			0.003		

Means followed by different letters within each variety are significantly different. BEG12-*Funneliformis mosseae*, BEG44-*Rhizophagus irregularis*, Har-*Trichoderma harzianum*, Asp-*Trichoderma asperellum*, BB-(BEG12+BEG44), HarS- (Har+Asp), HBB-(Har+BEG12+BEG44), C-control (un-inoculated).

Discussion

Arbuscular mycorrhizal fungi and *Trichoderma* spp substantially enhanced the shoot nutrient content in KVVU 27-1 and K80 across the three water levels with N, P and K contents greater in inoculated cowpea than their controls. This concurs with Metwally (2020) who recorded a higher concentration of shoot macronutrients after inoculating onion plants with different strains of AMF and *Trichoderma* spp.

The triple inoculation of *T. harzianum* + *F. mosseae* + *R. irregularis* (HBB) had greater influence on the nutrient uptake at the three water levels than when they were used singly. In support of these findings, Colla et al. (2015) established a higher concentration of minerals in the shoots and roots of lettuce, melon and pepper after co-inoculation with *G. intraradices* and *T. atroviride*. Yadav et al. (2015) also recorded a higher shoot and root P in *Hellinthus annulus* L. after co-inoculation of *G. mosseae* with *T. viride* and *A. laevis*. In this study, *Trichoderma* spp elicits production of auxins or auxin-like hormones which enhances massive rooting. The increased root surface area necessitates the uptake of nutrients leading to growth. Other studies on cacao (Tchameni et al., 2011), and onions (Metwally and Al-Amri, 2019) support these findings. However, it is worth noting that not all combinations of AMF and *Trichoderma* spp elicit synergistic responses in plants. In some studies, it was reported that the combination of AMF and *Trichoderma* reduced the root colonization percentage which reduced plant performance (Dehariya et al., 2015). Such decrease in root colonization could be due to fungal competition for nutrients and space.

The dual inoculation of *T. harzianum* + *T. asperellum* (HarS) influenced the highest nutrient uptake at various soil water levels. Halifu et al. (2019) also combined

Trichoderma isolates (*T. harzianum* and *T. virens*) and recorded an increased rhizospheric N and P that led to increase in biomass and seedling height of *Pinus sylvestris*. Detailed studies have shown that *Trichoderma* strains secrete organic acids which dissolve the less mobile minerals including P and Zn and enables them to be absorbed and utilized by plants. (Li et al., 2015).

The co-inoculation of the AMF species (BB) caused the highest N and K contents in K80 at 60 % soil water level. Kundu et al. (2013) concurs with these results as he recorded an increased macronutrients after inoculating a mixture of four AMF isolates on sweet potatoe. Studies have shown that a high AMF diversity in the soil creates greater fungal communities with broader benefits that complement each other benefiting the plant in terms of growth.

The effect of single inoculations on nutrient uptake was not outstanding yet it was significant. The shoot nutrient levels in the inoculated cowpea was greater than their controls. *T. harzianum* elicited greater response in the absorption of nutrients and was host-specific. At mild water stress (60%), *T. harzianum* caused the greatest shoot K uptake in KVVU 27-1. This increase in the shoot nutrient level is attributed to production of organic acids which chelate K from K-bearing minerals. Studies have shown that just as phosphorous, K exists in complex, non-absorbable forms in the soil and therefore microorganisms including *Trichoderma* are able to convert them into absorbable forms for the benefit of the plant.

The AMF inoculants also increased the tissue N, P and K in the both cowpea varieties above controls. Yaseen et al. (2011) also recorded increased levels of N, P, K, Ca, and Mg in the cowpea shoots after inoculating them with several inoculants of AMF. The accumulation

of nutrients in mycorrhizal inoculated plants is due to the extension of the extraradical hyphae beyond the roots which provides a large surface area for absorption of nutrients. Other authors have argued that AMF enables the solubility of these nutrients which prior exist in insoluble forms to be easily absorbed by plant roots (Altomare *et al.*, 1999).

The role of soil water levels and their effect on fungal inoculants in the uptake of nutrients by cowpea was also underscored. It was noted that the nutrient content decreased with the increase in drought stress in all fungal treatments and control. This is supported by Zhang *et al.* (2014), who recorded reduced amounts of P and other nutrients in water stressed conditions. However, in this study the inoculated plants had a significantly higher nutrient content than the control at each water level. In the un-inoculated cowpea plants the nutrients reduced significantly regardless of the water level. Khoshmanzar *et al.* (2019) noted greater intake of N, P, K and Fe by tomatoe after inoculating them with different *Trichoderma* isolates. Abdel-salam *et al.* (2017), also recorded elevated nutrient contents in the shoots and roots of mycorrhizal inoculated rose plants than their controls under water stress. Similarly, Gholamhoseini *et al.* (2013) supports these results when he recorded increased nitrogen concentration in AMF inoculated sunflower under water stress conditions. AMF-plant relationships intensifies nutrient uptake by increasing the absorptive surface area through formation of extra radicle hyphae which go beyond the roots (Gholamhoseini *et al.*, 2013).

Conclusion

From the results, AMF and *Trichoderma* spp significantly influenced the uptake of N, P and K at the three water levels for both varieties. This led to increase in the nutrient levels in the inoculated than the un-inoculated cowpea plants. Combined inoculations of HarS, HBB

and BB enhanced more uptake of nutrients and canbe utilized to reclaim the parched and unused land for beneficial agricultural activities.

Recommendation

The fungal inoculants are recommended for use on agricultural soils instead of chemical fertilizers because they are affordable and also reduces soil and water pollution.

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