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Effects of Arbuscular Mycorrhiza Fungi (AMF) and mineral phosphorus addition on the performance of sorghum (*Sorghum biocolor*.L) in Sudan.

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ABSTRACT

A pot experiment was carried out to study the effect of two types of mycorrhiza isolated from sorghum(*Sorghum biocolor L*.), and onion (*Allium ceba*) crops as compared to the addition of triple superphosphate on the growth of sorghum grown on a clay soil from Khartoum State, Sudan. The experiment was conducted during June–August 2011 under shadow in the demonstration farm, Sudan University, College of agricultural Studies, Shambat "lat.(15o 40 N) long.(32^o 32E), Sudan. The mycorrhiza was added at the rate of 300 spores / pot, while the mineral fertilizer was applied at the recommended dose 0.03g/pot (40kg triple superphosphate/ha).The design adopted was a Completely Randomized Design(CRD) with three replications. Plant samples were taken after four weeks from sowing and at harvest(8 weeks from sowing) to determine root colonization by mycorrhiza, plant height, root length, shoot dry weight, and root dry weight, in addition to shoot phosphorus content, and spores density. The results indicated significant differences in root colonization after four weeks from sowing and at harvest, upon addition of the mycorrhiza isolated from sorghum compared to the one isolated from onion . The infection was depressed when phosphorus fertilizer (P) was added to both types of mycorrhiza .However, the addition of mycorrhiza alone was superior to the addition of mineral P with mycorrhiza and to the addition of P alone. The results also revealed significant differences in plant height, root length, shoot dry weight, and root dry weight. The best results were obtained upon the addition of the mycorrhiza isolated from sorghum, compared to that isolated from onion. But when phosphorus fertilizer was used with mycorrhiza, all the measured traits were depressed. Also, the results indicated significant differences in spores density. High density was obtained when the sorghum mycorrhiza was added alone, and with onion mycorrhiza when mineral P was added as compared with other treatments. The results revealed highly significant differences in shoot phosphorus , especially when the plants were inoculated with sorghum mycorrhiza , followed by onion mycorrhiza. Both treatments gave high shoot phosphorus content compared to other treatments and to the control treatment.

Key words: Mycorrhiza, biofertilizer, spore density, colonization, triple superphosphate

INTRODUCTION

Sorghum (*Sorghum biocolor* L.) , the origin of which is Ethiopia has spread to other parts of Africa, India, South East Asia, Australia and the United states (Skerman and Riveros, 1990). The crop is cultivated to varying extent in almost all tropical and subtropical areas of the world . It is quite resistant to drought so it is grown in areas where rainfall is insufficient. (Quinby and karper, 1951). Members of the sorghum family used for forage include forage sorghums, sudangrass and various hybrids .These sorghum cultivars have little value as direct marketable seed crops, but their value becomes apparent as they are marketed through livestock feeding as animal products (Pederson and Fritz, 2000). There is considerable variability in agronomic and nutritional quality traits among species, hybrids and varieties. it has been documented that forage sorghums have the potential to produce as much , and in some cases more ,dry matter than corn when grown with the same amount of water (Anderson and Guyer,1986).

In the Sudan, the most important commercial forage sorghum cultivars include Abu Sabein (*Sorghum bicolor* L), sudangrass (*S.sudanensis*) and the hybrid; pioneer (*Sorghum bicolor* XS.). These grass forages are grown in the period from March to October as summer crops (Elkarouri and Mansi, 1980).

During the last decades, the increased costs of fertilizer production coupled with the progressively increasing use of chemical fertilizers are adding to the cost of crop cultivation .In addition, chemical fertilizers are harmful when they persist in the soil and enter the food chain .Instead, an approach is adopted to introduce into the soil potential microorganisms; a practice known as inoculation, which is also known as biofertilizers. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers. The uses of biofertilizer in agriculture play an important role providing an economically viable level for achieving the ultimate goal to enhance productivity. (Elhassan, G. A. *et al* (2010).

Mycorrhizal fungi form a bridge between the roots and the soil and gather nutrients from the soil and give them to the roots (Peters, 2002). Mycorrhiza also offers several benefits to the host plant, including faster growth, improved nutrition (wilox.1996).

Infection of the root system by these fungi creates symbiotic (beneficial) relationships between the plant and the fungus. Upon root infection and colonization, Mycorrhizal fungi develop an external mycelium which is bridge connecting with the surrounding soil (Toro *et al*, 1997). One of the most dramatic effects of infection by Mycorrhizal fungi on the host plant is the increase phosphorus (P) uptake (koide 1991), mainly due to the ability of Mycorrhizal fungi to absorb phosphate from soil and transfer it to the host root (Asimi *et al* 1980) . `

The information regarding the presence of mycorrhiza in the rhizosphere of the most economical crops in Sudan is scanty. Their relative efficiency in increasing P uptake needs to be investigated.

This study was therefore carried out to isolate mycorrhizal fungi from the rhizosphere of some of the important economical crops grown in Sudan i.e. sorghum and onion, and to compare the efficiency of the isolates with the addition of mineral phosphorus at the recommended application rate on sorghum growth traits, in an attempt to be able to recommend the use of biofertilizers as an alternative to chemical fertilizers for sorghum.

MATERIALS AND METHODS

The study was conducted at Sudan University of Science &Technology, college of agricultural studies (Shambat) during June–August 2011 under shadow with simulated conditions. Sorghum was grown in a clay soil . The soils used in this experiment were air–dried at ambient laboratory temperature for 24 hrs.The samples were further crushed to pass a 2mm mesh sieve. The soils properties determined included ; pH, EC, soluble anions and cations, percent $CaCO_3$, total nitrogen, total phosphorus, and mechanical analysis.

Plants were grown in black plastic bags (20 cm diameter, 5 kg capacity) .The bags were filled with 4 kg of a sterilized soil. Drainage holes were made in the bottom of the bags using a sterile needle .The soils used in this experiment were sterilized in an autoclave at 121^0 C ,and 15 1b pressure, for 2 hrs.

The Arbuscular Mycorrhiza (AM) fungal inoculums used in this experiment was isolated from sorghum and onion crop grown at shambat by wet sieving method and decanting technique as described by (Hayman 1982). In this technique 50 g of rhizosphere soil were suspended in one litter of tap water. The sorghum seeds were surface sterilized using 2% sodium hypochlorite for 15 min (Vincent, 1970), and washed 3 times with sterilized tap water. The sterile seeds were then transferred to petri dishes and incubated at 25^0 C for three days. Six peregrinated sorghum seeds were then planted in each pot and 300 spores of AMF inoculums were added to the designated pots. The inocula from AM fungi were earlier isolated either from the rhizosphere of onion or sorghum plants.

Three replications were made for each treatment. Both AM inoculum were added in two separate treatments to which was added the recommended dose of P as superphosphate (0.03g/pot)as recommended by Farah & Eastin (1988). A treatment was included to which P was added at the recommended dose without inoculum. A control treatment was also included without P-fertilization and without inoculum addition.

With 6 treatments, 3 replications, a total of 18 pots were laid in a completely randomized design. The pots were transferred to a shade at SUST greenhouse facilities. The pots were weekly rerandomized.

The growth period continued for 8 weeks after which the plants were sampled two times; after 4 weeks and after 8 weeks when the plants were harvested. Sampling was done by uprooting the plants. The roots were washed thoroughly using tap water. Plant height and root length were measured. Both shoots and roots were dried in an oven at 72^0 C for 48 hrs. , and dry weights for both were recorded. Available P was measured using a spectrophotometer .Mycorrhizal colonization was tested according to the grid line-intersect method described by Giovannetti and Mosse (1980), and by Trouvelot *et al*. (1986). Root samples were washed and cut into 1 cm length. The root samples were cleared in 10% KOH at 90°C for 15 min and washed in a sieve (45µm) under running water. Cleared roots were stained with 0.05% trypan blue in lactoglycerol at 121°C for 15 min. Thirty pieces of stained roots from each sample were mounted on glass slides to evaluate root colonization by AM fungi .

After 8 weeks from transplanting, the soil samples were collected and wet-sieved for spores density determination as described by An *et al*. (1990). Spores of AM fungi were separated from 100 g of each soil sample from the root zones of sorghum crop by wet sieving and 50% sucrose centrifugation. After centrifugation, spores in the supernatant were poured on the 45 µm sieve and washed with tap water to remove the sucrose and were further kept in petri dishes. Spores were counted under a stereomicroscope.

The results of treatments were statistically analyzed using, MSTATC. Mean separation was done using Duncan multiple range test,(Duncan 1955).

RESULTS

The soil chemical and physical properties of the soils used in this study is shown in used had an ECe of 2.1 DS/m, pH 7.6,total N content of 0.04%, available phosphorus 1.4ppm,$CaCO_3$, 6.5%, and CEC (43 cmole/kg (Table 1). The results are presented in tables (2-8).

Table 1: Chemical and Physical Properties of the Soils used in the Study

Soil property	Depth 0-30 cm
ECe(m mohs/cm)	1.7
pH(saturated soil paste)	7.6
Ca+Mg meq /1	9.6
Na (meq /1)	8.7
K (meq /1)	0.15
Cl (meq /1)	0.06
HCO3(meq/1)	1.9
Caco3 (%)	6.5
N (%)	0.04
P(ppm)	2.4
O.C (%)	0.7
O.M (%)	1.1
C/N	17
Sand (%)	11
Clay (%)	50
Silt (%)	39
Texture class	Clay soil
CEC(cmole/kg)	43
SAR	4

Inoculation with Vesicular Arbuscular Mycorrhiza(VAM) improved most of the measured traits compared to the uninoculated control. All of the measured parameters were improved by the time of sampling i.e. 37, 222, 39, 218,71 and 32% increase in plant height, shoot dry weight, root length, root dry weight, Shoot phosphorus content , and colonization percent, respectively.

Effect of treatments on plant height: After four weeks from sowing, the highest plant height, which could be attributed to mycorrhizal positive effect was observed in sorghum plants inoculated with sorghum mycorrhiza, followed by plants inoculated with onion mycorrhiza (table 2).The lowest height , however, was observed in the treatment which was inoculated with onion mycorrhiza to which mineral phosphorus was added.

However, after 8 weeks from sowing , the plants inoculated with onion mycorrhiza was superior compared to all other treatments and significantly outyielded all other treatments including even the plants inoculated with sorghum mycorrhiza.

Table 2: Effect of the treatments used on sorghum plant height at different sampling intervals(cm)

Treatment	Sampling interval	
	(4 weeks from sowing)	(8 weeks from sowing)
sorghum .Myco	56.0 a	76.3 b
Sorghum. Myco +P	49.0 cd	71.0 b
Onion. Myco	54.3 ab	78.7 a
Onion. Myco +P	41.0 e	63.0 c
(P)Recommended dose	45.3 de	71.3 b
Control	50.0 bc	60.7 c
C.V%	12.31	4.12

Effect of treatments on shoot dry weight:

The data presented in table (3) show the effect of treatments on shoot dry weight.

Analysis of variance indicated significant differences between sorghum mycorrhiza and onion mycorhiza over both treatments to which mineral phosphorus was added , and the control treatment. Both mycorrhizal treatments to which mineral phosphorus was added improved shoot dry weights over both the control treatment and to which mineral phosphorus was added.

Table 3: Effect of the treatments used on sorghum shoots dry weights at different sampling intervals(g)

Treatments	Sampling interval	
	(4 weeks from sowing)	(8 weeks from sowing)
sorghum .Myco	3.1a	10.4a
Sorghum. Myco +P	2.5ab	6.5c
Onion. Myco	2.8a	8.9a
Onion. Myco +P	2.6ab	8.8a
(P)Recommended dose	2.1b	7.1b
Control	1.8b	6.3b
C.V%	31.9	19.0

*Means having the same letters within each column are not significantly different at the
0.05 level of probability by the Duncan Multiple Range Test

Effect of treatments on root length: After 4 weeks from sowing, sorghum plants inoculated with mycorrhiza isolated from sorghum significantly increased root length of sorghum plants compared to plants inoculated with onion mycorhiza (table 4). Both treatments increased root length when compared to the control treatment and the one to which mineral phosphorus was added at the recommended dose. The same trend was almost observed at harvest time (8weeks from sowing).

Table 4: Effect of the treatments used on sorghum root length at different sampling intervals(cm)

Treatments	Sampling interval	
	(4 weeks from sowing)	(8 weeks from sowing)
sorghum .Myco	17.3a	25.6a
Sorghum. Myco +P	15.6a	20.0c
Onion. Myco	13.6b	16.6d
Onion. Myco +P	12.3bc	20.3b
(P)Recommended dose	12.0c	17.6d
Control	11.3bc	14.0e
C.V%	13.2	14.9

*Means having the same letters within each column are not significantly different at the 0.05 level of probability by the Duncan Multiple Range Test.

Effect of treatments on root dry weight

Treatments inoculated with mycorrhiza (both sorghum and onion) significantly improved root dry weights compared to the uninoculated controls and to the treatment to which mineral phosphorus was added.

At harvest time , the highest root length was observed with plants inoculated with sorghum mycorhiza compared to all other treatments (table 5).

Table 5 : Effect of the treatments used on sorghum root dry weights at different sampling intervals(g)

Treatments	Sampling interval	
	(4 weeks from sowing)	(8 weeks from sowing)
sorghum .Myco	1.4b	5.0a
Sorghum. Myco +P	1.5a	4.a
Onion. Myco	1.1bc	2.5c
Onion. Myco +P	0.9c	3.9b
(P)Recommended dose	0.9bc	2.7c
Control	0.8c	2.9c
C.V%	43	35.3

*Means having the same letters within each column are not significantly different at the 0.05 level of probability by the Duncan Multiple Range Test.

Effects of treatments on root colonization: In the non-mycorrhizal treatments, sorghum roots remained free of AM fungal colonization.

Table (6) present the effect of treatments on root colonization percentage of sorghum plant, which showed significant improvement after 8 weeks from sowing (32%), Sorghum mycorhiza inoculum significantly increased colonization percentage compared to other treatments, followed by the mycorrhiza isolated from onion. Addition of mineral phosphorus, however, decreased colonization percentage in both sources of mycorrhiza i.e. sorghum and onion.

Table 6: Effect of the treatments used on colonization percentage at different sampling intervals(%)

.Treatments	Sampling interval	
	(4 weeks from sowing)	(8 weeks from sowing)
Sorghum. Myco	65.0a	78.3a
sorghum Myco.+P	44.0b	56.6b
Onion .Myco.	50.0b	77.3a
Onion. Myco.+P	48.3b	62.0b
C.V%	14.0	10.4

* Means having the same letters within each column are not significantly different at the
0.05 level of probability by the Duncan Multiple Range Test.

Effect of treatments on tissue phosphorus: Inoculation with VAM significantly increased the shoot phosphorus content of sorghum crop. Compared to the uniculated plant table (7) . The best values of phosphorus content were observed with the sorghum mycorrhiza, followed by onion mycorrhiza . Both were superior to the treatments to which both mycorrhiza and mineral P were added and to the control treatment.

Table 7: Effect of treatments used on tissue phosphorus at harvest time (mg/plant)

Treatments	Harvest Time (8 weeks from sowing)
sorghum .Myco	0.74a
Sorghum. Myco +P	0.39b
Onion. Myco	0.45b
Onion. Myco +P	0.36c
(P)Recommended dose	0.39b
Control	0.28c
C.V%	16.3

*Means having the same letters within each column are not significantly different at the 0.05 level of probability by the Duncan Multiple Range Test.

Effect of treatments on spores density: Table (8).which presents the effect of treatment on spores density showed that the best spores density was observed with sorghum mycorrhiza treatment compared to all other treatments.

Table 8:Effect of treatments on spores density /100g soil

Treatments	Harvest Time (8 weeks from sowing)
sorghum .Myco	480.0a
Sorghum. Myco +P	386.6b
Onion. Myco	216.6c
Onion. Myco +P	463.3 ab
C.V%	23.44

*Means having the same letters within each column are not significantly different at the 0.05 level of probability by the Duncan Multiple Range Test.

DISCUSSIONS

The present investigation was conducted to study the possibility of using VAM as biofertilizer to sorghum crop. The use of VAM enhanced growth and development of the plant. The results indicated significant differences between treatments. However, the best colonization was observed in plant root infected with mycorrhiza isolated from sorghum ,followed by mycorrhiza isolated from onion. Both treatments were superior than the mycorrhiza treatment upon addition of phosphorus. Phosphorus addition lead to reduction in mycorrhizal infection. This result was in agreement with (kodi 1991), where the addition of mineral phosphorus depressed most of the measured traits including growth parameters.

Also the results indicated a significant increase in spores density. The highest spore's density was reported with sorghum mycorrhizal inoculums. High spores density in the soil lead to an increase in root infection. It is apparent from these findings that the mycorrhiza isolated from sorghum is superior in improving the measured growth traits and in increasing P uptake by sorghum plants. This could be attributed to genetic compatibility similar to what had been reported in Rhizobium- legume symbiosis (Graham, 1982).

The shoot phosphorus content was significantly increased by VA mycorrhiza. The best values were observed in sorghum mycorrhizal inoculums followed by onion mycorrhizal inoculum. Similar results were reported by (kodi 1991). The data also agree with the findings of Asmah, A. E. (1995), and Subramanian et. al,(2006)., who reported that plants colonized by AM fungi were much more efficient in taking soil P than nonVAM plants. Also several studies had unequivocally demonstrated that plants colonized by AM fungi were much more efficient in uptake of P (Fitter, A. H. 1998, Goicoechea, *et al*.,1997,. Subramanian, K. S. and Charest, C. 1999).

Future studies are needed to field evaluate these findings and to search more for the crop-mycorrhiza interactions, especially with the sorghum plant as a food security crop in Sudan.

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