Review Article

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Effects of Inoculation with Indigenous mycorrhizal isolates and mineral fertilization on the performance of sorghum *(Sorghum vulgare*) under greenhouse condition in Sudan.

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This work was carried out to investigate the effect of indigenous strains of mycorrhizal Fungi and fertilization on sorghum (*Sorghum vulgare*) under greenhouse conditions. Upon harvest (three months from sowing), the data collected included shoot and root dry weights, plant height, color rating, root colonization, tissue phosphorus, tissue nitrogen ,and tissue potassium. Significant Differences were recorded between all treatments. The addition of local mycorrhiza greatly enhanced plant growth traits in the sorghum plant. Tissue P was increased which is reflected in the dry weight of the roots. Fertilization with mineral phosphorus significantly influenced some of the plant growth traits measured.

Key words: indigenous mycorrhiza, fertilization, sorghum.

Millions of people throughout the world, including Africa, Asia, and other semiarid regions depend on sorghum as a staple crop. It represents the fifth most abundant crop worldwide (C. F. Klopfenstein *et al*., 1995). In many households, sorghum is the primary source for energy, protein, vitamins, and minerals.

In recent years, biofertilizers have emerged as promising components of the integrated nutrient supply system in agriculture. Among biofertilizers benefiting the cereal crop production are *Azotobacter, Azospirillum, cyanobacteria,,* P-solublizing microorganisms and mycorrhizae (Kulik, 1995).

Most of Sudanese soils are deficient in the two major macronutrients; nitrogen and phosphorus. Moreover, when they are applied as fertilizers besides their high cost, mineral nitrogen fertilizers are subject to losses by volatilization and leaching, while mineral phosphorus is reported by many workers to be subject to adsorption, especially in the high calcareous soils dominating Sudan.

Investigations in Sudan showed that plant inoculated with mycorrhizal fungi increased dry matter, phosphorus and nitrogen content, and-in case of legumes-better nodulation and nitrogen fixation (Atabani, 1988). Plants inoculated with locally- isolated mycorrhizal spp. showed benefits equivalent to and sometimes greater than plants inoculated with introduced VA mycorrhizal fungi (Mahadi, 1993). Govindarajulu *et al.* (2005) stated that roots of many plant species are naturally colonized by arbuscular mycorrhizal (AM) fungi, which are ubiquitous in soil. AM can directly uptake inorganic nitrogen from the soil and transfer it to the host plant. AM plants have been reported to improve nutrition of other macronutrients including nitrogen and potassium (Liu *et al*., 2002). Also, it had been reported that higher concentrations of K were found in mycorrhizal than in non-mycorrhizal plants, in addition to enhancing both growth and yield of inoculated plants (Mohamed *et al.,* 2008).

This research was therefore conduced in an attempt to elucidate the existence of indigenous mycorrhiza in Sudanese soils with certain crops of economical importance (Alfalfa, sugarcane and date palm), and to look into their efficiency in improving the nutrition of one of the most consumed crop by Sudanese people; sorghum (*Sorghum vulgare*).

MATERIALS AND METHODS

Soil samples collection:

Soils and roots samples of three crop plants (Alfalfa, sugarcane and date palm) were collected from the rhizosphere (0-30cm) depth from two different sites in Sudan; Northern State and Khartoum state. Five replications were made for each collection site. Soil samples were further kept in a refrigerator adjusted at 4^0C until used.

Isolation of VAM spores:

The spores were isolated by wet sieving and decanting method (Gerdemann, and Nicholson, 1963). Modifications were made where 50 g of representative soil sample were drawn from each site and suspended in 1000 ml of tap water and stirred thoroughly. The suspension was then allowed to stand for 15 minutes and passed through a series of sieves with different sizes i.e. (1 mm , 500µm, 250µm, 125µm, 53 µm and 45 µm) arranged in a descending order of their mesh size. The spores on the six sieves were transferred to a 250 ml conical flask and kept for further use.

Inoculation of VAM spore:

Trap culture:

For propagation of the isolated spores, , Sudan grass was planted in a sandy soil washed by hydrochloric acid. Eighty seeds were surface sterilized by H_2O_2 (3%) for 15 minutes, The inocula , which had been isolated from date palm, alfalfa, and sugarcane were added at the rate of 300 spore/pot where the sudangrass seeds were grown. Five replications were made for each of the three treatments. A nutrient solution (Ashiton, 40%) was added to the grown sudangrass every week. The experiment duration was three months.

Greenhouse experiment:

The study was conducted in a greenhouse in August 2011, at Sudan University of science and technology, college of agricultural studies. (LAT: 15º 40'N, LONG: 32º 32'E and ALT.: 380 M). The temperature was adjusted at 20 °C during the night and 25 °C during the day. Relative humidity was maintained constantly at 55 %. The seeds of sorghum (Mogod variety) were obtained from the Agricultural Research Corporation (Gadarif station), were surface sterilized by H_2O_2 (3%) for 15 minutes and washed three times by sterilized water. The sterilized seeds were then transferred to Petri dishes and incubated at $30C^o$ for two days using an incubator model (LIB030M). Eight seeds were aseptically added per pot. The plants were grown in black plastic bags (20cm diameters, five kilogram capacity). The bags were filled with 4kg of a sterilized soil. Drainage holes were made in the bottom of the bags using a sterile needle. The pots were irrigated immediately with a sterile tap water. Prior to the filling of the pots, the top soil was sieved using a 2 mm mesh sieve and then steam sterilized at 121 ^{o} C and 15bar/inch^2 pressure, for 2 hours using an autoclave. This was to eliminate native arbuscular mycorrhizae fungi propagules as well as other microorganisms. For all treatments, seeds were placed at 5-cm depth from the soil surface. For the AMF treatments, 200g and 400g of the appropriate AMF inoculm was placed in the soil over which the seeds were planted. As described by Azcon *et al*. (1991). For the fertilizers treatments urea and superphosphate were used at the recommended dose (Abdelgadir *et al*., 2010). Nine treatments were included using different spore counts for inoculation. A control without mycorrhizal inoculation and without fertilization was added as check treatment.Soils were maintained at moisture holding capacity by periodically adding water. Pots were kept in the greenhouse and repositioned once a week.

After three months from sowing, plants colors were rated and plants heights were measured. Root dry weight, top dry weight and root colonization were also conducted.

RESULTS AND DISCUSSION

Table 1 presents the chemical and physical properties of the soils used in the greenhouse experiment. The soils used were clay soils, deficient in nitrogen and phosphorus

Table 2. The effect of isolated indigenous mycorrhizal fungi and chemical fertilizers on the measured plant growth traits.

Treatments*	Plant high Cm/plant	Top dry weight gm/plant	Root dry weight gm/plant	Root colonization%	Color rating
MS200	96.5^{DE}	1.0^{CD}	0.66^{CD}	71.0^{C}	2.6^{B}
MD200	102.8^{DC}	1.1^{B}	0.83^{B}	75.0^{B}	3.0^{B}
MA200	106.0^{C}	1.1^{CB}	0.70^{CD}	71.6^{C}	3.0^{B}
MS400	107.6^{C}	1.2^{B}	0.63^{D}	75.6^{B}	2.6^{B}
MD400	125.6^{A}	1.4^{A}	0.83^{B}	88.3^{A}	3.0^{B}
MA400	116.1^{B}	1.2^{B}	0.66^{CD}	75.3^{B}	3.0^{B}
P	85.1^{F}	1.2^{B}	0.96^{A}	0.0^{D}	3.0^{B}
N	95.1^{E}	1.2^{B}	0.76^{CB}	0.0^{D}	4.0^{A}
Control	87.5^{F}	0.9^{D}	0.66^{CD}	0.0^{D}	3.0^{B}
CV	3.9	6.5	8.5	3.1	14.8

*Means with the same letter within the same column are not significantly different at 5% probability by the Duncan multiple range test.

Table1. Chemical and physical properties of the soil used in the study of the greenhouse experiment.

Soil property	Value
ECe (Ds/m)	1.4
pH(Paste)	7.7
Na (Meq/l)	12
K(Meq/l)	0.3
Ca+Mg (Meq/l)	7.5
CO_3 (Meq/l)	0.0
HCO_3 (Meq/l)	3.4
Cl (Meq/l)	0.08
SO_4 (Meq/l)	16.4
P (ppm)	2.7
N (%)	0.04
O.C (%)	0.7
C/N	17
Sand%	11
Silt%	34
Clay%	54
Texture class	Clay soil
SAR	6

Plant growth traits

The results tabulated in (table 2) indicated significant differences between treatments. However, the best plant height was observed with mycorrhiza isolated from date palm (MD400), followed by mycorrhiza isolated from alfalfa (MA400) and sugarcane (MS400), also with the best top dry weight (1.4), root dry weight all compared with control. The best value of color rating observed with treatment of (N).

The highest value of root colonization and spore density were showed with mycorrhiza isolated from date palm (MD400). These results agree with Mahadi, (1993) and Mohamed *et al,* (2008).

MS200: Indigenous mycorrhiza isolate from Sugar cane (200g/pot), MD200: Indigenous mycorrhiza isolate from Date palm (200g/pot), MA200: Indigenous mycorrhiza isolate from Alfalfa (200g/pot), MS400: Indigenous mycorrhiza isolate from sugar cane (400g/pot), MD400: Indigenous mycorrhiza isolate from Date palm (200g/pot), MA400: Indigenous mycorrhiza isolate from Alfalfa(400g/pot), P: Recommended dose of superphosphate, N:Recommended dose of urea and C: Control w/o inoculation w/o fertilization.

Plant tissue mineral composition

Table 3 presents plant tissue mineral composition of the macronutrients i.e. N, P, and K. The statistical analysis revealed highly significant differences between all treatments. The treatment of (MD400) recorded high tissue nitrogen percentage per plant, phosphorus and potassium compared with the control treatments.

It has been well documented that mycorrhizal colonization can have significant impacts on plant tissue mineral composition (Govindarajulu *et al.* (2005), Hodge *et al*. (2001), and Liu *et al*. (2002).

Table 3. The effects of indigenous mycorrhizal isolates and mineral fertilizers on plant tissue content of major nutrients.

Treatments	N%	P%	K%
MS200	1.2^{d}	0.0001^{e}	0.006^{def}
MD200	2.0^{b}	0.0003^{c}	0.010^{abc}
MA200	1.5^{cd}	0.0001^{e}	0.011^{a}
MS400	1.5 ^{cd}	0.0001^{e}	0.008^{bcd}
MD400	3.4^{a}	0.0006^{a}	0.011^{a}
MA400	2.0^{b}	0.0004^{b}	0.007 ^{cde}
P	1.6^{c}	0.0003^{c}	0.003^{f}
N	3.2^{a}	0.0003^{c}	0.005^{ef}
C	1.3 ^{cd}	0.0002^{d}	0.007^{cde}
CV	9.8	23.4	20.0

Root colonization

The statistical analysis showed highly significant differences between plants infected by the indigenous isolated mycorrhiza. The mycorrhiza isolated from date palm when applied at a dose of 400g/pot recorded high percentage of infection followed by mycorrhiza isolated from sugar cane and alfalfa when applied at the dose of 400g/pot. The rate of increase was 88.3%, 75.6%, and 75.3%, respectively). The application of the isolates at a dose of 400g/pot was superior in all to the application of 200g/pot (79.7vs 72.5%).

Table 4: Effect of indigenous mycorrhizal fungi isolated on sorghum root colonization and spore density.

Treatments	Root colonization%	Spore density
MS200	71.0^{c}	1183.3^{d}
MD200	75.0^{b}	1977.3^{c}
MA200	71.6^{c}	1279.3^{e}
MS400	75.6^{b}	1997.3^{c}
MD400	88.3^{a}	3867.0^{a}
MA400	75.3^{b}	2468.3^{b}
C.V%	3.1	1.5

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

It is apparent that the Mycorrhiza isolated from date palm rhizosphere (D400) outyielded all other isolates in plant height, tissue dry weight, and root colonization followed by mycorrhiza isolated from alfalfa (MA400), which also gave the highest color rating, both being significantly different from the addition of 200spore/plant. However, the mycorrhiza isolated from sugar cane (MS) at both inoculant levels was significantly lower than the two prementioned mycorrhizae (D) and (A), especially when added at the lower rate of 200g/pot. Increasing the dose of (MS) mycorrhizae from 200 to 400g/pot improved the measured traits significantly.

Addition of mineral fertilizers did not improve the measured Parameters except for root dry weight when P was added as superphosphate. Mineral nitrogen, on the other hand improved the color rating significantly over all other traits including all mycorrhizal stains added at the two rates.
Root colonization could therefore could be rated as:

MD400＞MS400＞MA400＞MD200＞MA200＞MS200.

All treatments, however improved the measured traits significantly than the control treatments.

This was reflected in N, P, and K content in plant tissue and resulted in significant increase in mineral content of the plants, and outyielded all other treatments except in nitrogen content when mineral fertilizer nitrogen was added.

Unexpectedly Tissue K% was increased. When MA mycorhizae were added at the rate of 200g/pot, and gave comparable figures to the addition of 400g/pot of the (MD) mycorrhizae.

Further research is needed to confirm such results in the field. Also more work is needed to classify the mycorrhizal isolates obtained from the rhizosphere of the different crops in Sudan, and test their efficiency to select the ones with superior performance to cater partially for the deficiency of phosphorus noticed with Sudanese soils,

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