Response of Sesame (Sesasum indicum L.) to Vesicular Arbuscular Mycorrhiza (VAM) and Mineral phosphorus Additions at different moisture regimes under greenhouse conditions in Sudan

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Abstract- This study was carried out during the year 2010-2011 to investigate the effect of soil properties on mycorrhizal spore density, and to find out the ameliorative effects of Vesicular Arbuscular Mycorrhizae (VAM) addition on different growth traits of sesame (*Sesamum indicum L.*); an important cash crop in Sudan economy using two mycorrhizal fungi isolated from date palm trees rhizosphere (from two locations in Khartoum State).VAM inoculation was compared with phosphorus chemical fertilization under different moisture conditions. The study was divided into two parts; a laboratory and a green house experiment. In the laboratory experiment, mycorrhiza from West Omdurman gave a superior spore density (1363 spore/50g soil), while other tested soils gave no significant differences in root infection. This was followed by Soba-2 and Soba-1. All soils have an alkaline reaction. Electrical conductivity in West Omdurman soils is higher than Soba soils. All soils have low CEC ranging from 6-19 cmole/Kg reflecting their low ability to retain soil nutrients. Soba soils are calcareous, while West Omdurman had low CaCO3. As regarding soil texture, both Soba-1 and West Omdurman soils are sandy, while Soba-2 had a clay texture, which might reflect the noticed variability in spore counts between the two soils. Available phosphorus values ranged between 1-2.9 ppm. In the greenhouse experiment, four moisture levels (0%, 20%, 40% and 60%) were used. At 20% moisture level, the data obtained revealed high superiority in all growth traits measured irrespective of the location. The data obtained demonstrated that spore density was higher in West Omdurman, but root infection percent was higher in Soba. The data obtained with the growth parameters were variable but without noticed differences.

Inoculation with mycorrhiza showed more efficiency, and were positively reflected in growth traits (plant height, leaf number, dry weight, tissue phosphorus and nitrogen) than addition of mineral phosphorus.

I. INTRODUCTION

Sudan has a wide range of tropical and continental climates with large daily and seasonal fluctuations in temperature, (Salih O. A. 2005).

The increased costs of fertilizer production coupled with the progressively increasing use of chemical fertilizers are adding to the cost of crop cultivation (Elhassan, G. A. *et.al* 2010). It is assumed that many of the biological products being offered to farmers contain beneficial microorganisms (bacteria and fungi) with the potential to promote plant health. (Laditi, M. A. *et. al* 2011).

The potential uses of biofertilizers in agriculture play an important role of providing an economically viable level for achieving the ultimate goal to enhance productivity. Soils in Sudan have moderate to poor mineral fertility. The main causes are the low content of nitrogen (N), available phosphorus (P) and sometimes potassium (K). (FAO 2006). Most soils in dry land areas are low in both plant-available water and soil nutrients and artificial fertilizers are rarely used by small-scale farmers in semi-arid Sudan. (Helen. T., and J. Ardö 2009). Arbuscular mycorrhizal fungi (AMF) are important components of agricultural ecosystems, and can directly influence the productivity of these systems. Unfortunately, conventional agricultural practices have been shown to adversely affect AM fungi.

Mycorrhizal fungi as a biofertilizer, form a bridge between the roots and the soil and gather nutrients from the soil and give them to the roots (Peters, 2002). The association (mycorrhizal fungi and plant) helps the plant obtain water, which is critical to plant survival and growth under dry conditions.

Water, being essential for plant growth and development and much concern has been expressed in the recent year towards increase in the area of arid regions of the world. Crop productivity in arid region depends on the length, magnitude and stage of the plant that is affected by moisture stress. (Havargi, R. S. 2007)

Plants colonized by mycorrhizal fungi have been shown to deplete soil water more thoroughly than non-mycorrhizal plants as the shoots of plants with AMF usually have a larger biomass (more evaporative leaf surface area) than non- AMF plants. Also, the root systems of plants with AMF are often more finely divided and thus have more absorptive surface area (Farahani, A. *et.al.,* 2008). Other cultural practices optimum for increasing productivity are also lacking in Sudan. Scanty information is available regarding water requirements since the crop is usually grown under rainfed areas of the country.

This study was therefore conducted to verify the efficiency of local isolates of vesicular arbuscular mycorrhizal (VAM)

fungi on sesame production. Thus, the specific objectives of this research were therefore to:

1. Investigate on the possibility of improving sesame growth using VAM fungi.
2. Evaluate the effects of soil moisture content on sesame growth and mycorrhizal Infection percentages of sesame roots.
3. Compare between biological fertilizer (mycorrhizae) and chemical fertilizer on sesame growth.
4. Know the effects of soil properties on VAM fungi.

II. Materials and Methods

Laboratory experiment:

Two soils with different texture were investigated to explore the existence of vesicular arbuscular mycorrhizal fungi (VAM) in date palm trees grown in Soba and West Omdurman (Sudan). In Soba (15°31'36.34"N and 32°36'11.92"E), date palm is the main flora in the farm, Varity Majhol and Barhy and were irrigated from the White Nile.

Some spots in the farm were calcareous. (Plate 1)

In West Omdurman (15°37'10.70"N and 32°13'57.53"E) also the vegetation type is date palm and is irrigated from a tube well. Chicken manure and urea fertilizer were being used. Depending on the homogeneity of the sites, samples were collected randomly from the two sites. (Plate 2). The collected samples were divided into two category; one for monitoring mycorrhiza to estimate spore density. Also, root samples were taken to observe root colonization under a compound microscope. The other sample was taken for soil analysis, which was performed by the personnel at the soil and water science laboratory of the department of Soil and Water Sciences, College of Agricultural Studies, Sudan University of Science and Technology, using standard techniques.

EC, pH and soluble salts were determined on paste saturation extract (Ritchard, 1954) using a pH meter (model 3510), EC meter (model M35). Na and K were estimated using direct flame photometry in the soil extract (Flame photometer (Model 410)).CaCO3 wasestimated using a calcimeter, Model (Eijkelkamp). Total nitrogen was performed usingthe Kjeldahl method (Ryan *et. al.*, 2001). Organic carbon was determined by the Walkley and Black method (1934). For available phosphorous, O'lsen (1954) method was used using a spectrophotometer model (6305). The amount of exchangeable potassium was estimated by the use of direct flame photometry in the soil extract (Ryan *et al*., 1996). Soil texture was determined using Particle Size Determination (Pipette Method), and textural classes were defined using USDA textural triangle.

For Mycorrhiza spore extraction the method described by (Gerdemann and Nicholson,

1963) was used. Mycorrhiza spore density was estimated by the method described in

23 enumeration of spores by (An *et al.*, 1990). For root staining, colonization, the methods described by (Giovannetti and Mosse, 1980 and Chabaud *et al.*, 2006) was followed.

Greenhouse experiment:

A greenhouse experiment was conducted in soil and water science department, college of agricultural Studies, Sudan University of science and technology, to investigate the response of sesame to mineral phosphorus addition and to the application of VAM fungi under different moisture regimes under controlled conditions. Four Kilograms of soils collected from the university field, were packed in plastic bags and sterilized using an autoclave adjusted at (121°C and 15bar pressure) for 30 minutes twice every 24 hours for 48 hour. Seeds of sesame (*Sesamum indicum L*., var. Khidir) obtained from the Agricultural

Research Center Wad Medani were surface sterilized using Hydrogen peroxide (3% H2O2) and incubated at 28 °C for 3 – 4 days in an incubator Model (LIB030M). Selected 6 pregerminated seeds were then planted in the plastic bags at 5-cm depth. The pots were irrigated immediately using sterilized tap water to avoid contamination.

The pot experiment consisted of four stress treatments *viz.*, control (normal irrigation), moderate stress (60% field capacity), high stress (40 % field capacity) and severe stress

(20 % field capacity). The treatments details are given below:

Stress 1 (0%): normal irrigation, water added at the rate of 500 ml / pot.

Stress 2 (20% F.C.): severe stress, water was added at a rate of 749.6 ml / pot.

Stress 3 (40% F.C.): high stress, water was added at a rate of 1499.2 ml / pot.

Stress 4 (60% F.C.): moderate stress, water was added at a rate of 2248.8 ml / pot.

Control (C) without phosphorus fertilization or Mycorrhizal fungal spores was included.

Mycorrhizal fungal spores isolated from Soba soil were added at the rate of 30 spores/pot. Mycorrhizal fungal spores isolated from West Omdurman soils added at the rate of 300 spores/pot and mineral phosphorus was added as recommended by ARC (Agricultural Research Corporation, Sudan), where triple superphosphate was added at the rate of (20 Kg superphosphate/hectare i.e. 0.04 g/pot). The bags were placed in the green house facility of the College of Agricultural Studies at Shambat with the temperature adjusted during the three months growing period at 20 °C at night and 25°C during the day. The pots were irrigated on alternative days, and 24 regularly weighed to keep them at the designated field capacity. The pots containing the soils were weighed daily during the experiment. Based on the loss of weight of the pots, water was added regularly to maintain the required moisture stress in different stress treatments. Every treatment was replicated three times. All the pots were arranged in a completely randomized design in the greenhouse facility. The pots were rerandomized weekly.

At harvest: three months from sowing, the color of the shoots was rated, leaf numbers were counted, and stem length was measured per plant. Plant shoots were dried at 720C for 48 hours and weighed. Tissue nitrogen and tissue phosphorus were determined using standard analytical methods Ryan *et al.*, (1996). Spore density and root colonization were determined using the method of (Gerdemann and Nicholson, 1963) and (Giovannetti and Mosse, 1980 and Chabaud *et al.*, 2006) respectively.

Statistical Analysis:

MSTATC program was used for statistical analysis of the obtained results.

Plate (1): Soba's farm picture from satellite.

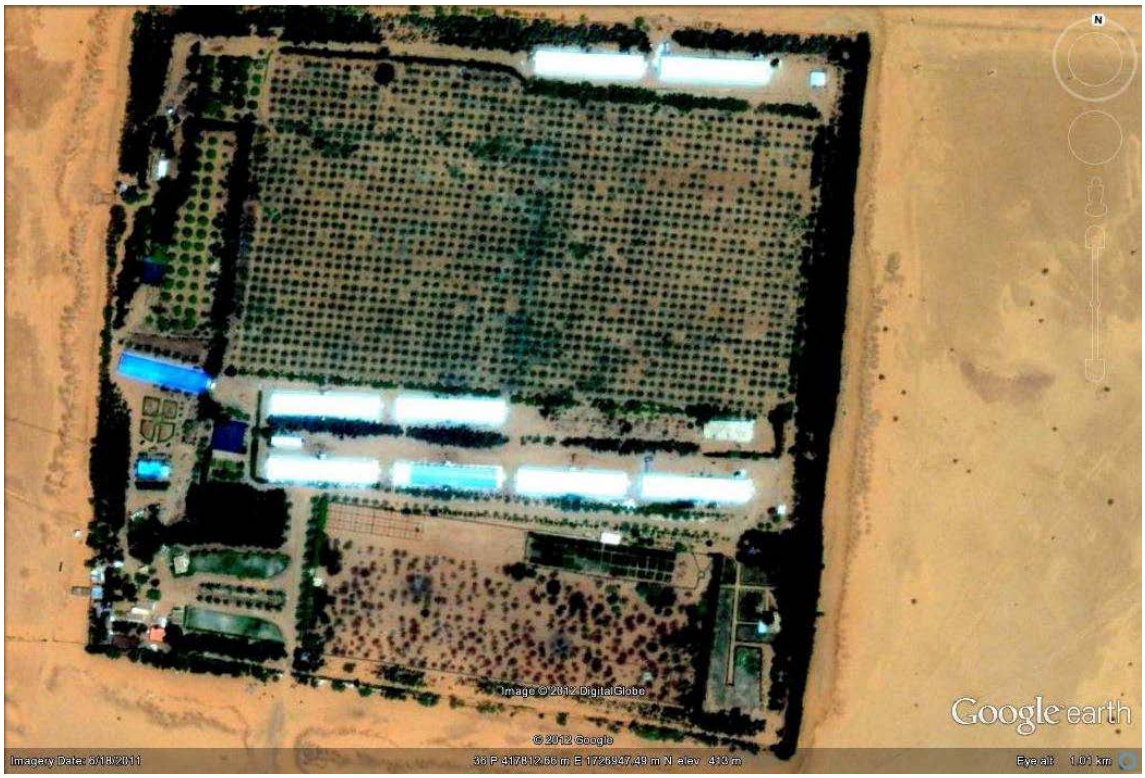

Plate (2): West Omdurman picture from satellite.

III. Results and Discussion

Laboratory work

All data are presented in table (1-10). The data regarding soil chemical and physical properties are presented in table (1) and (2) Correlation matrices revealed several of the determined soil chemical and physical properties. Both E.Ce and pH measured for Soba-1 soils showed a positive correlation with spore density. In contrast, a negative correlation was observed with Soba-2 and West Omdurman Soils .This could be an indicator of presence of different strains of mycorrhiza in Sudanese soils irrespective of the crops associated with them.

The determined soluble salts showed a positive correlation with Soba-1 soils ranging from (0.6-0.9). However, $CaCO_3$ gave a negative correlation (-0.79). All measured soluble salts in Soba-2 soils, on the other hand were positively correlated with spore density except potassium. $CaCO_3$ also showed a negative correlation with spore density.

Correlation with exchangeable cations was negative for all traits measured. Also a positive correlation was consistently obtained with moisture percentage, organic carbon and with clay content. Exemptions are noted with total nitrogen and silt percent which revealed a negative correlation with spore density in both Soba soils. Also, a positive correlation was reported with available phosphorus with Soba-2 soils, while both Soba-2 and West Omdurman soils showed a negative correlation. All the tested soils have an alkaline reaction (pH 8). Electrical conductivity in West Omdurman is higher than Soba soils. All soils had low CEC reflecting their ability to retain soil nutrients. Soba soils are calcareous having 15% $CaCO_3$, while West Omdurman had low $CaCO_3$.

Organic carbon ranged between 1 - 2 and total nitrogen values were in the range of 0.007- 0.1. And so the available phosphorus values ranging between 1-2.9 ppm. As regarding soil texture, both Soba-1 and West Omdurman soils are sandy, while Soba-2 has a clay texture, which might reflect the noticed variability in spore counts between the two soils.

This necessitates further work to classify the kinds of mycorrhiza dominating the rhizosphere of the crops investigated. The data also revealed a significant variation in spore density with West Omdurman giving a higher spore density than both Soba soils (1363 spores/50g soil vs. 600). However, this difference was not reflected in the percent of root infection noted with the date palms grown in all soils.

Greenhouse experiment:

The data presented in table (3) show the properties of the soil used in the greenhouse pot experiment. The soils are clay loam in texture, had alkaline reaction, moderate in cation exchangeable capacity, high saturation percentage and low in nitrogen, organic carbon and phosphorus.

The data presented in table (4) shows the spore density as influenced by moisture. The data are rather erratic. No trend is apparent whether moisture is adjusted at 0 or 60% F.C. No significant variations were also noted with the mycorrhiza isolated from West Omdurman or Soba. Similar results were reported with the percentage root infection table (5), although the isolated mycorrhiza from Soba soils seemed to have achieved a relatively higher root infection percent at the normal irrigation practices and at 20% moisture stress treatment.

Table (6) presents the data obtained when mineral phosphorus was applied at the recommended dose (20 Kg superphosphate/hectare) together with the mycorrhizal isolates from Soba and West Omdurman No apparent differences were observed whether with the treatments added or with the adjusted levels of moisture, except the obvious trend towards the increase in tissue phosphorus upon addition of the mycorrhiza isolated from West Omdurman, both when normal irrigation was applied, or at 20% moisture in contrast to other treatments. It's worth mentioning that most of the sesame plants grown at high moisture stress (40 and 60%) could not resist the desiccation and died.

Similar results could be noticed regarding tissue nitrogen as tabulated in table (7) the mycorrhiza isolated from West Omdurman showed higher tissue nitrogen at 20% moisture stress in contrast to other treatments. This might represent a further evidence needs to be explored in future studies regarding the variations reported above.

When assessing the tissue dry weights of the harvested sesame plants, table (8) revealed that a significant increase with West Omdurman mycorrhiza at both the normal irrigation practice and at 20% moisture stress. However, the control plants at 20% moisture stress gave a significantly higher tissue weight and out yielded all other treatments at all levels of stress. This was clearly demonstrated in table (9) with respect to the plants leaves numbers.

Plant height data is presented in table (10). Significant variations were observed upon treatment of the sesame plants with mineral phosphorus. Similar results could be noticed with the application of mycorrhiza isolated from both Soba soils, both values being at 20% moisture stress.

Although the data obtained in the research conducted gave erratic results which could be justified as due to the short duration of the experiment, general conclusions could be drawn, in agreement with the findings of the research conducted by (Runjin, 1989; Ruiz- Lozano *et al.*, 1995; Al-Karaki, 1998; Auge *et al.*, 1987; Amico *et al.*, 2002; Hardie and Leyton, 1981 and Subramanian and Charest, 1998). All researchers confirmed the benefits of using mycorrhiza in increasing water use efficiency in different crops. The mycorrhizal plants were found to be more water-use efficient than nonmycorrhizal plants. Also, the plants they used, including barely, wheat, tomato, and roses had higher shoot and root dry matter than nonmycorrhizal plants regardless of water stress level.

The plants they experimented with, also had higher shoot and root dry matter than nonmycorrhizal plants regardless of water stress level. AMF colonization increased total P uptake by all the genotypes they used regardless of water-stress level. The improved growth, yield and nutrient uptake in their plants demonstrated the potential of mycorrhizal inoculation to reduce the effects of drought stress on plants grown under field conditions in semiarid areas of the world. Their results also indicated that AM plants had greater tolerance to drought stress than non-AM plants as biomass and grain yields were higher in mycorrhizal than nonmycorrhizal plots irrespective of soil moisture. The mycorrhizal plants were found to have higher shoot P and Fe concentration than nonmycorrhizal plants at all samplings regardless of soil moisture conditions. This comes in agreement with the general observations noted in this study as

related to the relative efficiency of the isolated mycorrhiza from Sudanese soils.

This is evident from the work of Fidelibus *et al.*, 2001 who tested the hypothesis that growth and water-use characteristic of AM plants when they used AM fungal isolates of different geographic origins.

Table (1): chemical and physical properties of study area*

Samples	ECe	pH	CEC cmole	Soil particles distribution			Saturation %	Ex.Ch Cations meq/100gsoil			O.C %	N meq/100g soil	P ppm
				Sand %	Silt %	Clay %		Na	K	Ca+Mg			
Soba-1	0.6[b]	8.3[a]	19[a]	72[a]	20[b]	8[b]	36[ab]	0.9[a]	0.2[a]	17.6[a]	2.0[a]	0.1[a]	2.2[a]
**CORR	0.948525	0.66309	0.59127	-0.13174	-0.165	0.15017	0.92434	0.31670	-0.6631	0.66460	0.905936	-0.3167	0.463891
Soba-2	0.6[b]	8.2[b]	17[b]	36[b]	42[a]	22[a]	38[a]	1.1[a]	0.2[a]	15.4[b]	2.3[a]	0.1[a]	1.0[a]
**CORR	-0.40829	-0.96525	-0.40829	0.829453	-0.995	0.96524	0.99268	0.75264	-0.26133	-0.52833	0.985842	-0.26133	-0.9666
West Omdurman	1.9[a]	8.0[c]	6[c]	67[a]	24[b]	9[b]	33[b]	0.4[b]	0.1[b]	5.8[c]	1.0[b]	0.07[a]	2.9[a]
**CORR	-0.969	-0.96271	0.80127	-0.74929	0.0070	0.91120	0.6308	0.05949	-0.69847	0.82693	0.999975	0.968998	-0.32217

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

Table (2): Spore density and root infection of mycorrhiza isolated from two sites (Soba and West Omdurman)*

Samples	Mycorrhizal Root Infection%	Mycorrhizal spore density spores/50g soil
Soba-1	63[a]	459[b]
**CORR	0.186396	-
Soba-2	49[a]	914[ab]
**CORR	0.530862	-
West Omdurman	52[a]	1363[a]
**CORR	0.998639	-

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

Table (3): Shambat soil chemical and physical properties: *

ECe	pH	Soluble Cations			Soluble Anions			SAR
		Na meq/L	K meq/L	Ca+Mg meq/L	CO_3 meq/L	HCO_3 meq/L	Cl meq/L	
1.4	7.7	10.7	0.3	7.8	0.0	3.2	3.6	6
Soil particles distribution			Textural class	CEC Cmol/Kg soil	Moisture Content	Saturation%		
Sand%	Silt%	Clay%						
11	34	55	Clay loam	55	3	80		
Exchangeable Cations			N%	O.C %	P ppm	$CaCO_3$ %		
Na meq/100g soil	K meq/100g soil	Ca+Mg meq/100g soil						
4.3	0.1	50.6	0.04	0.1	3.0	0.1		

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

Table (4): The effect of two strains of indigenous VAM and mineral phosphate additions on the spore density of sesame cultivar as influenced by moisture: *

Treatments	Moisture stress	
	0%	20%
Mycorrhizae soba	442[a]	588[a]
Mycorrhizae W.O	529[a]	652[a]

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

Table (5): The effect of two strains of indigenous VAM and mineral phosphate additions on the root infection% of sesame cultivar as influenced by moisture: *

Treatments	Moisture stress	
	0%	20%
Mycorrhizae soba	12^{a}	3^{c}
Mycorrhizae W.O	8^{b}	3^{c}

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

Table (6): The effect of two strains of indigenous VAM and mineral phosphate addition on the tissue phosphorus of sesame cultivar as influenced by moisture: *

Treatments	Moisture stress	
	0%	20%
Control	0.010^{bc}	0.020^{a}
Mineral P	0.013^{b}	0.020^{a}
Mycorrhizae soba	0.013^{b}	0.020^{a}
Mycorrhizae W.O	0.007^{c}	0.023^{a}

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

Table (7): The effect of two strains of indigenous VAM and mineral phosphate additions on the tissue nitrogen of sesame cultivar as influenced by moisture: *

Treatments	Moisture stress	
	0%	20%
Control	0.150^{c}	0.177^{c}
Mineral P	0.100^{cd}	0.300^{b}
Mycorrhizae soba	0.037^{d}	0.367^{ab}
Mycorrhizae W.O	0.030^{d}	0.433^{a}

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

Table (8): The effect of two strains of indigenous VAM and mineral phosphate additions on the dry weight of sesame cultivar as influenced by moisture: *

Treatments	Moisture stress	
	0%	20%
Control	0.233^{d}	1.200^{b}
Mineral P	0.400^{d}	0.433^{d}
Mycorrhizae soba	0.300^{d}	0.767^{c}
Mycorrhizae W.O	0.467^{d}	2.000^{a}

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

Table (9): The effect of two strains of indigenous VAM and mineral phosphate additions on the leaf number of sesame cultivar as influenced by moisture: *

Treatments	Moisture stress	
	0%	20%
Control	8^{d}	26^{b}
Mineral P	9^{cd}	18^{bc}
Mycorrhizae soba	11^{cd}	45^{a}
Mycorrhizae W.O	14^{cd}	27^{b}

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

Table (10): The effect of two strains of indigenous VAM and mineral phosphate additions on the shoot length/ plant of sesame cultivar as influenced by moisture: *

Treatments	Moisture stress	
	0%	20%
Control	18.3[c]	33.0[ab]
Mineral P	18.4[c]	15.7[c]
Mycorrhizae soba	18.3[c]	40.9[a]
Mycorrhizae W.O	15.8[c]	29.4[b]

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

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