Review Article

©*ISISnet Publishers*

Bioscience Research, 10(2): 65-70, 2013
Available online at **www.isisn.org**
Print ISSN: 1811-9506
Online ISSN: 2218-3973

Response of groundnut cultivars to inoculation with indigenous AM Fungi and alien *Rhizobium* strain under greenhouse conditions.

M.M. Alsamowal[1], M. A. Hadad[2] and G. A. Elhassan[3]

[1] Graduate Student, College of Agricultural Studies, SUST, **Sudan**
[2] Associate Professor of Soil Microbiology, College of Agricultural Studies, SUST, **Sudan**
[3] Professor of Soil Microbiology, College of Agricultural Studies, SUST, **Sudan**
***Corresponding author**

Legumes may respond to non-rhizobial inoculants such as Arbuscular Mycorrhizal (AM) fungi either through an effect on plant growth or, in addition, through an effect on the function of the legume *Bradyrhizobium* symbiosis. AM fungi and *Bradyrhizobium* positively affected peanut plant growth yield and nutrients uptake in greenhouse and field. The performance of the AMF alone or in combinations with *Bradyrhizobium* strains was significantly better than that of the bacteria alone in terms plant height; top dry weight and root dry weight.

Key words: indigenous mycorrhiza, fertilization, groundnut.

Most of Sudanese soils are deficient of the two major macronutrients; nitrogen and phosphorus. Moreover, when they are applied as fertilizers besides its high cost, they are subject to losses by volatilization and leaching of nitrogen and fixation of phosphorus. Inoculation with *rhizobia* was found to improve nodulation and yield of many leguminous crops, such as Faba bean (Mukhtar and Abunib, 1988); chickpea, (Ibrahim and Salih, 1980); dry bean (Gobara, 1988). 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 (Mahdi, 2004). Plants inoculated with locally isolated mycorrhizal spp. showed benefits equivalent to, and sometimes greater than, plants inoculated with introduced VA mycorrhizal fungi (Mahdi, 1993). Ahmed (2000) reviewed the use of VAM fungi as a biofertilizer in Sudan and suggested that VAM fungi have a great potentiality as a biofertilizer where soils were found to be very poor in available phosphorus.

During the last decades, the increased costs of fertilizers 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 it adopted to introduce into the soil potential microorganism, a practice known as inoculation. The inoculants were also known as biofertilizers. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers. The microorganisms, which are potential biofertilizers, are symbiotic and non-symbiotic nitrogen fixing microorganisms, phosphorous solubilizing microorganisms and silicate bacteria. 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. (Elhassan et al. 2010).

In Sudan, however, scanty information is available regarding the use of biofertilizers despite the pioneer work conducted by many authors (Mahdi, 1993; Ahmed, 200,; Mahdi, 2004).

Therefore, this work was conducted with the following objectives: 1) To isolate local mycorrhiza from Sudanese soils associated with the most crops of economical importance e.g. date palm, sugar cane and alfalfa, and test their efficiency in alleviating phosphorus deficiency continuously reported in Sudanese soils. 2) To test the efficiency of *Rrhizobium* strains inoculation, alien alone and when mixed with mycorrhizal inoculum in improving the yield of selected crops of economical importance.

MATERIALS AND METHODS

Soil samples collection:

Soil and root samples of three crop plants (Alfalfa, sugar cane 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 with roots of respective plant species were collected and placed in plastic bags and kept refrigerated until further use.

Isolation of VAM spores:

The spores were isolated by wet sieving and decanting method (Gerdemann, and Nicholson, 1963), with the following modifications. Fifty grams of representative soil sample were drawn from each site and suspended in 1000 ml of tap water and stirred thoroughly. The suspension

was allowed to stand for 15 minutes and then passed through a series of sieves 1 mm size, 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 250 ml conical flasks and kept at 4^0C for future experiments.

Inoculation of VAM spore:

Trap culture:

For propagation of the isolated spores, an experiment was conducted at the college of Agricultural Studies, Sudan University of Science and Technology. Sudan grass was planted in a sandy soil washed by hydrochloric acid (10%). Eighty seeds were surface sterilized by H_2O_2 (3%) for 15 minutes. The inoculum which had been isolated from plants and trees roots rhizosphere (date palm, alfalfa, sugarcane) was added at the rate of 3000 spores/pot. Five replications were made for each of the three treatments. Also, a nutrient solution (Ashiton 40%) was added to the pots every week. The experiment duration was three months. Count of spores followed and the root were stained using trypan blue to count infection percentage.

Rhizobium strains:

The *Rhizobium* strain (ICRSAT 7001) was obtained from Natural Resource and Environmental Research Institute, International Center for Agricultural Research, Sudan. Erlenmeyer flasks containing 200 mls of sterile medium (YEMB,Vincent, 1970) were inoculated with the *Rhizobim* strain under aseptic conditions. The flasks were placed on an orbital shaker at 200 rpm at ambient temperature for three days. (Howieson et al. 2004). Serial dilutions were made to count the *Rhizobium* cells/ml. Approximately (300×10^8 CFU)/ml were obtained.

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 35 °C during the day. Relative humidity was maintained constantly at 55 %. Seeds of sorghum (Mogod) variety were obtained from The Agricultural Research Corporation (Algadarif station). Maize seeds (*Zea maize*);Hidiba1 variety and groundnut (Ahmadi) variety were obtained from the Agricultural Research Corporation (Wad Medani Station, Sudan), were surface sterilized by H_2O_2 (3%) for 15 minutes and washed three times by sterile water. The sterilized seeds were then transferred to petri dishes and incubated at 30 ^{o}C for four days. Eight seeds were aseptically added per pot. The plants were grown in black plastic bags (20cm diameters, five kilogram capacity), which were previously filled with sterile 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. 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 inoculum was placed in the soil over which the seeds were planted. For the *Rhizobium* treatments, one ml of the *Rhizobium* inoculum culture was added to the sown seeds, as previously described by

Azcon et al. (1991). For the AMF treatments + *Rhizobium* treatments 200g and 400g of the AMF inoculum were added to the sown seeds. One ml of the designated *Rhizobium* inoculum, whether alien or native was also added. For the fertilizers treatments, urea, superphosphate and ammonium sulphate were used at the recommended dose (Farah and Eastin 1988). A control w/o inoculation w/o fertilization was also included.

Plants were thinned to five per pot following seedlings emergence. Plants colors were rated. Pots were randomized in the greenhouse and repositioned once a week.

Plant samples and Tissue analysis:

Samples from each plot were taken randomly after one measure of nodules dry weight. At the end of the growing period (3 month from sowing), plant samples were further dried to a constant weight in a forced-air oven at 72 C^{o} for 48 h. The top dry and root weights were determined.

Spore density determination:

At harvest, mycorrhizal spore extraction of the soils were accessed by taking 50 g of soil sample from all the AM treatment pots (including the bulk soil sample used for the experiment after sterilization) after thoroughly mixing. Sucrose density centrifugation gradient was applied at 2000 rpm for 3 minutes (An, *et, al*., 1990). The spores were thereafter examined and counted under a dissecting microscope.

Mycorrhizal infection determination:

The degree of mycorrhiza infection was assessed as described by (Giovannetti and Mosse, 1980, and Trouvelot *et. al*., 1986).

Statistical analysis:

The design used in the greenhouse experiment was completely randomized design with three replicates. Statistical analysis was conducted using, (SAS) program. Mean separation was done using Duncan multiple range test, (Duncan 1955).

RESULTS AND DISCUSSION

The results are tabulated in tables 1 to 5. The chemical, physical and biological properties of the soils used in the greenhouse experiment are shown in table (1).The effect of the different treatments on growth traits and root colonization and spores density counts are presented in table (2) and (3). Number and mass of main and lateral root nodules are presented in table (4).Tissue mineral composition is presented in table (5).

Effects of treatments on growth traits

The highest plant height, which could be attributed to mycorrhizal positive effect was observed in groundnut plants inoculated with (M100), (M50) and treatment (10^{4}x), followed by plants inoculated with *Rhizobium* (10^{4}). Also the groundnut plants inoculated with mixture mycorrhiza (M100) were superior regarding top dry weight compared to all other treatments, and significantly out yielded all other treatments including even the plants inoculated with *Rhizobium* and the uninoculated control. The highest roots dry weight, however, was observed in the treatment, which was inoculated with (M100). On the other hand, highest value of color rating was observed with plants inoculated with *Rhizobium* interaction with minerals added and treatment of urea recommended dose. The highest root colonization and spore density observed in treatment of (M100) followed by other treatments inoculated by local mycorrhizal fungi isolated. These results agree with bean Mukhtar and Abunib (1988), Ibrahim and Salih, (1980), Gobara, (1988). Mahadi and Atabani (1988) and Mahadi, (1993). Shown in table (2-3).

Effects of *treatments* on number and mass of nodules:

The statistical analysis showed that the peanut mycorhiza mixture with bradyrhizobium inoculum significantly increased number and mass of nodules compared to both other treatments (104), (108). The data also agree with the findings of Osman and Mohamed (1996).

Effect of local mycorrhiza isolated, alien rhizobium strain and fertilization on peanut plant content of total nitrogen (N2), phosphorus (P) and potassium (K) uptake.

Treatment mixture of bradyrhizobium with mycorrhiza (104x) significantly improved absorption of macro elements (N2, P, K) compared to the uninoculated controls and other treatments. These results could be attributed to mycorrhizal positive infection. Treatments both (104x) and urea recommended dose observed highest value of total nitrogen content compared with control,

Table (1). Chemical, physical and biological properties of the soils used in the greenhouse experiment.

Soil property									
pH	Ece	Soluble Cations (Meq/l)			Soluble Anions (Meq/l)				SAR
Paste	(Ds/m)	Na	K	Ca+Mg	CO_3	HCO_3	Cl	SO_4	
7.9	2.8	11.0	0.1	8.6	0.0	2.8	0.09	16.8	5
P (ppm)	N (%)	O.C (%)	C/N	Soil particles distribution %			Texture class		Spore density 50g/soil
4.1	0.08	0.8	10.0	Sand	Silt	Clay	Clay soil		76
				9.0	31.0	60.0			

Table (2). Effect of local mycorrhiza, alien *Rhizobium* strains and fertilization on groundnuts plant height, top dry weight, root dry weight, root colonization and color rating.

Treatments	Plant height cm/plant	Top dry weight gm/plant	Root dry weight g/plant	Color rating*
M50	18.3^{ab}	1.2^{cb}	1.2^{bcd}	2.6^{cd}
M100	18.6^{ab}	1.4^{a}	1.8^{a}	3.0^{bc}
10^4	17.0^{bc}	1.2^{b}	1.3^{ed}	3.3^{abc}
10^8	16.3^{c}	0.7^{fg}	0.9^{e}	2.6^{cd}
10^4x	19.6^{a}	1.2^{b}	1.3^{b}	3.0^{bc}
N	15.6^{c}	1.1^{c}	1.4^{b}	4.0^{a}
P	15.6^{c}	0.8^{ef}	1.2^{bc}	2.6^{cd}
C	15.3^{c}	0.6^{g}	1.0^{cde}	2.0^{c}
C.V%	5.8	6.3	11.8	14.3

*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.

* Color was rated as: 4:dark green; 3: green; 2: yellow green, and 1: yellow

Table (3). Effect of indigenous mycorrhizal fungi on groundnuts root colonization and spore density.

Treatments	Root colonization%	Spore density
M50	46.6^{b}	5304.0^{b}
M100	54.0^{a}	10628.0^{a}
10^4x	46.3^{b}	5320.67^{b}
C.V%	**23.0**	**0.24**

*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.

Table (4). Effects of *Rhizobium* inoculation on the number and mass of nodules of groundnuts.

Treatments	Number of nodules basic	Mass of nodules basic Mg	Number of nodules lateral	Mass of nodules lateral Mg
10^4	46^{b}	30.6^{b}	46^{b}	46.6^{b}
10^8	32^{c}	19.3^{c}	33^{c}	33.0^{c}
10^4x	52^{a}	33.3^{a}	53^{a}	53.3^{a}
C.V%	**16**	**15.6**	**8.9**	**8.9**

*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.

Table (5). Effect of local mycorrhiza, *Rhizobium* strain and fertilization on groundnuts tissue mineral composition.

Treatments	N%	P%	K%
M50	1.2^{d}	0.00001^{d}	0.04^{ab}
M100	2.2^{b}	0.00003^{bc}	0.06^{a}
10^4	1.4^{cd}	0.00002^{c}	0.02^{abc}
10^8	1.4^{cd}	0.00001^{d}	0.02^{abc}
10^4x	3.5^{a}	0.00007^{a}	0.06^{a}
N	3.4^{a}	0.00003^{bc}	0.04^{ab}
P	1.7^{c}	0.00004^{b}	0.04^{ab}
C	1.4^{c}	0.00002^{c}	0.02^{abc}
C.V%	7.8	30	22.5

*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.

Table (6). Abbreviations used for treatments in the current study

Abbreviations	
M50	Mixture of local mycorrhizal isolated from sugarcane,date palm and alfalfa (200g/pot).
M100	Mixture of local mycorrhizal isolated from sugarcane,date palm and alfalfa (400g/pot).
10^4	Number of cells/ml used with the alien *Rhizobium* strain (ICRISAT7001).
10^8	Number of cells/ml used with the alien *Rhizobium* strain (ICRISAT7001)..
10^4x	Number of cells/ml used with the alien Rhizobium strain (ICRISAT7001). Upon mixing mixture with local mycorrhizae.
N	Urea used at the recommended dose.
P	Supephosphate used at the recommended dose.
C	Control w/o inoculation w/o fertilization.

also the statistical analysis system showed that there is was no significant differences between treatments among the potassium content in peanut plants. These all results similar to Artursson et al. (2006).

REFERENCES

Ahmed, F.E., S.O. Yagoub and E.A.E. Elsheikh, (2000). Effects of mycorrhizal inoculation on the nodulation, mycorrhizal infection and yield components of faba bean grown ynder two different watering regimes. U.K. J. Agric. Sci., 8: 107-117.

An, Z.Q., Hendrix, J.W, Hershman, D.E. and G.T. Henson, (1990). Evaluation of the “most probable number” (MPN) and wet-sieving methods for determining soil-bornepopulations of endogonaceous mycorrhizal fungi. Mycologia 82: 516-581.

Artursson, V., Finlay, R.D. and Jansson, J.K. (2006). Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ. Microbiol. 8, 1–10.

Azcón R, Rubio R and JM. Barea, (1991) Selective interactions between different species of mycorrhizal fungi and Rhizobium meliloti strains, and their effects on growth, N2-fixation (15N) and nutrition of Medicago sativa L. New Phytol 117 (3):399-404.

Days, P.R, (1956). Report of the committee on physical analysis 1954-55. Soil sci. soc. Amer. Proc. 20:167-169.

Duncan , B.O. (1955). Multiple Range test and Multiple F – test .Biometrics 11, 1-42.

Elhassan, G. A., Migdam E. Abdelgani, Awad G. Osman, Somaya S. Mohamed and Belgees S. Abdelgadir, (2010) Potential Production and Application of Biofertilizers in Sudan, p1,2.

Farah, S. M. and Eastin, J. D. (1988). Effect of N, P and ridges on sorghum. ARC Annual Report, Gezira, Sudan.

Gerdemann, J.W.,and T. H. Niclson, (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol.Soc., 46,235-244.

Giovannetti, M. and , B. Mosse, (1980). An evaluation of techniques to measure vescular-arabuscular infection in root. New-phyto 84:489-500.

Gobara, L.A.M. (1988). The effects of inoculation, phosphorus and Nitrogen fertilization on growth of *Haricot bean (Phaseolus vulgaris).* M.SC. (Agric.) thesis, univ. of Khartoum.

Howieson, J. and Ballard, R. (2004). Optimising the legume symbiosis in

stressful and competitive environments within southern Australia - some contemporary thoughts. Soil Biology and Biochemistry 36, 1261-1273.

Ibrahim, M.H. and F.A. Salih, (1980). Chickpea nodulation under saline conditions in Northern Sudan. International Chickpea Newsletter 2, 16-17.

Mahadi, M. A. (1993). Mycorrhizal Associations, Journal of Agriculture Science, Univ. of Khartoum.vol.1,No.1,pp.143-145.

Mahdi, A.A., A.B. Nur Eldaim and A.A. Arbab, (2004). Influence of mycorrhizal inoculation form of phosphate fertilization and watering regime on nodulation and yield of soybean. U.K. J. Agric. Sci., 12: 345-356.

Mukhtar, N.O. and Abu Naib, S.A. (1988). Biological nitrogen fixation by faba bean, Ientil and chickpea in the traditional versus the new areas of the Sudan. pp. 321-329. In: Nitrogen Fixation by legumes in Mediterranean Agriculture. Beck, D.P. and Materonm L.A. (eds.), Martinus Nijhoff.

Olsen, S. R, and C.V. Cole, (1954). Estimation of available phosphorus in soil by extraction with $NaHCO_3$ W.S. Dept. Agric. Circular No. 393.

Osman, A.G. and S.S. Mohamed, (1996). Effect of inoculation with Rhizobium strain TAL 1400 on the symbiotic properties of faba bean in Khartoum State. AlBuhuth J., 5: 126-139.

Ritchard, A. (1954). Diagnosis and improvement of saline and alkali soil.Hand book No. 60. USDA.

Standard Analytical Methods of the Member Companies of Corn Refiners Association, Inc. (1995). Pennsylvania Avenue, N. W. Washington, DC, 2006,USA.

Trouvelot A, Kough J, Gianinazzi-Pearson (1986). Measuring the rate of VA mycorrhization of root systems. Research Methods for Estimating havinga functional significance. In: Gianinazzi-Pearson V and Gianinazzi S (Eds), Proceedings of the 1st European Symposium on Mycorrhizae: Physiological and Genetically Aspects of Mycorrhizae, Dijón, INRA, Paris, pp. 217-222.