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The effect of local mycorrhiza isolated, *Rhizobium* and fertilization on *zea mays* and groundnuts plant productivity under field condition in Sudan

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Abstract

The study was performed to test the effects of isolates local mycorrhizal fungi from date palm, alfalfa and sugarcane plants on zea maize plants growth traits (plant height, top dry weight, root dry weight, color rating, root colonization and tissue content of nitrogen, potassium and phosphorus). Also, the effects local and alien strains of *Rhizobium*, and isolated local mycorrhiza (VAM) on groundnut plant growth traits were studied. The use of VAM enhanced growth and development of maize plants. VAM isolated from date palm enhanced the performance of maize plants and improved all traits tested over the uninoculated plants and controls. AM fungi and *Rhizobium* positively affected groundnuts plant growth traits and nutrient. The performance of the AMF alone or in combinations with *Rhizobium* strains was significantly better than that of the bacteria alone in terms of plant height, top dry weight and root dry weight.

Key words: Mycorrhiza,*Rhizobium*, Zea mays, Groundnut.

Introduction

Maize (Zea mays L.), also known as Indian corn and simply as corn, is an important crop worldwide, not only because it is the third cereal after wheat and rice and more important than either as a forage crop, but also because of its numerous uses and because of the shortage of its supply compared with the increasing demand (Babiker, 1999).

In Sudan, maize is considered a minor crop and it is normally grown in Kordofan, Darfur and Southern States or in small irrigated areas in the Northern states, with average production of about 0.697 ton/ha (FAO, 2005). Groundnut (*Arachis hypogea*) is an important oil crop grown in central, eastern and western regions of Sudan. It is mainly produced for its seed oil, which is an important cooking oil in Sudan. It is believed to be introduced to western Sudan by West African immigrants about two to

three hundred years ago. Farmers' varieties previously grown in that area were of the runner type locally known as “Abu Hibailat”, which is a type believed to be available at present only in some remote and isolated areas, with a high risk of disappearance. Fortunately, some few accessions were collected from the traditional runner type from South Kordofan state in 2004. Groundnut material collected so far showed a considerable variation in growth habit, seed size and colour (The Higher Council for Environment and Natural Resources, 2009).

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, silicate bacteria and mycorrhizal fungi. The potential uses of biofertilizers in agriculture play an important role of providing an economically viable level for achieving the ultimate goal to enhance economical crops productivity. (Elhassan *et al.*, 2010).

Investigations in Sudan showed that plant inoculated with mycorrhizal fungi enhance nodule formation and dry matter (Mahdi *et al*., 2004). Also (Ahmed *et al*.,1998). Mycorrhizal inoculation significantly increased nodule number, nodule dry weight, flower set, pod production and seed yield compared to non-mycorrhizal plants under both watering regimes. Other study by (Ahmed *et al*., 1998) both VAM inoculation and phosphorus fertilization significantly increased the shoot and root fresh and dry weights, number of nodules and dry weight of nodules under normal and saline conditions. However, work on maize improvement in Sudan is limited and only three cultivars have been released. These are var.113, a selection from local material; Giza 2 and Mogtamaa 45.

The objectives of this study was

1. To test indigenous mycorrhizal fungi isolated from Sudanese soils associated with the most crops of economical importance.
2. To test the efficiency of the isolates in improving the yield of *Zea mays* plant.

3. To test the efficiency of *Rhizobium* strains inoculation, both introduced and indigenous alone and when mixed with mycorrhizal inoculum in improving the yield of groundnut.

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 from depth 0-30cm of two different sites of 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 at 4^0C until used.

Isolation of VAM spores:

The spores were isolated by wet sieving and decanting method (Gerdemann, and Nicholson, 1963). 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 of 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 a 250 ml conical flask.

Inoculation of VAM spores:

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 sandy soil washed by hydrochloric acid. Eighty seeds were surface sterilized by H_2O_2 (30%) for 15 minutes. The inoculums which had been isolated from plants and trees (Date palm, alfalfa, sugarcane) was used at the rate of 3000 spore for each and inoculated pots. A nutrient solution (Ashiton,40%) was added to the soil every week. Five replications were made or each treatments. The experiment duration was three months.

Field Work:

The field work was conducted in 2012 during the rainy season (July to October) on a clay soil at Shambat, Sudan. Total rainfall during the rainy season at this location was approximately 95mm and supplemental irrigation is required. Groundnut was hand planted in head on ridges 70 cm apart with an in–row spacing of 20cm. Two seeds were placed in each hole. The plot size was 3×2 m, with four ridges per plot. Maize seeds were also hand planted on ridges 70 cm apart with an in–row spacing of 25cm. Three seeds were placed in each hole. The plot size was 3×2 m, with four ridges per plot. Planting dates were on the 6th and 7th of July, 2012. Plots were irrigated approximately every two weeks depending on rainfall. All plots were hand weeded during the season to eliminate competition from weeds.

The treatments applied for the groundnut experiment were as follows: ICRISAT 7001 (104/g), ENNRI 24(104/g), urea at the recommended dose (43kg/ha.), superphosphate at the recommended dose (43kg/ha.), ammonium sulphate at the recommended dose (85kg/ha.), mixture of mycorrhizae (date palm, alfalfa and sugarcane); 300spores, mixture mycorrhizae (date palm, alfalfa and sugarcane); 600 spores, alfalfa mycorrhiza, 300 spores and a control w/o inoculation w/o fertilization.

Zea maize treatments were as follows: sugar cane mycorrhizal inoculum (300 spores), sugar cane mycorrhizal inoculum (600 spores), date palm mycorrhizal inoculum (300 spores), date palm mycorrhizal inoculum (600 spores), alfalfa mycorrhizal inoculum (300 spores), alflfa mycorrhizal inoculum (600 spores), superphosphate at the recommended dose (43kg/ha.), superphosphate at two rates (86kg/ha.), and a control w/o inoculation w/o fertilization.

Plant samples:

The samples from each plot were taken randomly after one month, two months and three months.

Soil analysis:

Soils from the plots were chemically analyzed before experiment onset. Samples were air-dried in an open bench at ambient temperature in the soil laboratory for 72 hours. Soil pH was determined using a pH-meter, model (3510) with glass electrode according to the method of (Richard, 1954). Electrical conductivity of the saturation extract (ECe) was determined using electrical resistance bridge model (M35) as

described by (Richard, 1954). Soluble cations (Na+, K+ and Ca+++Mg++) and soluble anions (CO3, HCO3-, Cl- and SO4--) were determined according to (Richard, 1954). Sodium adsorption ratio was calculated as follows:

$$SAR = Na / (\sqrt{Ca + Mg/2})$$

Total nitrogen was determined by kjeldhal method (*Ryan et. al*., 2001). Available phosphorus was determined using Olsen method (1954). Organic carbon was determined by Walkley and Black (1934).

Mechanical analysis was determined using the hydrometer method (Days, 1956). Texture class was determined according to the American system using textural triangle.

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 plots 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.

Tissue analysis:

The weight and count of nodules were made at the flowering stage. Plant samples were further dried to a constant weight in a forced-air oven at 72oC for 48 h. The top dry and root weights were determined. For ash content of the different samples, the standard method as described by (Ryan, *et. al*., 1996). Plant samples were extracted using 10 ml hydrochloric acid (5N) in a sand bath for 15 minutes. The extract was filtered and made to 50- ml volume. potassium were determined using a Flame photometer (Ryan *et. al*., 1996). .Phosphorus was determined using a Specterophotometer (Ryan *et. al*., 1996). Nitrogen content determined using kjeldal method (Ryan et. al., 1996).

Mycorrhizal infection determination:

Mycorrhiza staining was initiated by heating the root in 10 % KOH for 1h at 90Co. After which they were rinsed in water and soaked in 1 % HCl for 5 minutes. The staining solution, The Trypan blue stain used was prepared by mixing 250 ml of glycerol, 250 ml lactic acid, 300 ml of distilled water and 0.05g of trypan blue in water bath, at 90Co for 1 hour. The degree of mycorrhiza infection was assessed by

spreading the root samples evenly on a grid - line intercept plate and observed under the microscope. The total number of spores and infected roots intersecting the grids were counted following the grid-line intersect methods of (Giovannetti and Mosse, 1980, and Trouvelot *et. al*., 1986).

Statistical analysis:

The field experiment design was randomize block design with three replicates. Statistical analysis was conducted using (SAS) program. Mean separation was done using Duncan multiple range test, (Duncan 1955).

Results and Discussions

Table 1 shows the chemical and physical properties of the soils used in the experiment. The other measured plant traits were tabulated in tables (2-13)

Table (1). Chemical, physical and biological soil properties in field experiment

Soil property									
pH Paste	**Ece** (Ds/m)	**Soluble Cations** (Meq/l)			**Soluble Anions** (Meq/l)				**SAR**
		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**
				Sand	**Silt**	**Clay**			
4.1	**0.08**	**0.8**	**10.0**	**9.0**	**31.0**	**60.0**	Clay soil		**76**

1. ***Zea maize:***

1.1 Effect of local mycorrhizal isolates and phosphorus fertilizer on plant height and color rating:

The results indicated a significant increase in plant height. The highest value of plant height was reported with date palm mycorrhizal inoculums both treatments (MD1 and MD2), followed by sugarcane mycorrhizal inoculums. It is apparent from these findings that the mycorrhiza isolated from date palm is superior in improving the measured plant height. Also the treatment of mycorrhizal inoculums (MD1) significantly improved zea maize color rating, and there were no significant differences between other treatments These results agree with Mohamed *et. al*., (2008).

Table (2). Effect of the treatments used on *Zea maize* plant height and color rating at different sampling intervals.

Treatments	plant height cm/plant			Color rating		
	(1) Month	(2) Month	(3) Month	(1) Month	(2) Month	(3) Month
MS1	72.6^{d}	113.3de	132.0e	2.7^{ab}	3.0^{a}	3.0^{c}
MS2	78.6^{c}	121.0c	142.3c	3.0^{a}	3.0^{a}	3.0^{c}
MA1	66.3^{ef}	111.6e	125.6f	2.3^{ab}	3.0^{a}	3.0^{c}
MA2	68.3^{e}	114.0d	133.63d	3.0^{a}	3.0^{a}	3.3^{b}
MD1	82.0^{b}	141.6b	167.6b	3.0^{a}	2.7^{ab}	4.0^{a}
MD2	88.3^{a}	159.0a	176.6a	2.7^{a}	2.0^{c}	3.0^{c}
P	64.3^{f}	106.0g	123.0fg	2.7^{a}	3.0^{a}	3.0^{c}
P (2)	63.3^{f}	108.6f	137.6d	2.7^{a}	2.3^{bc}	3.0^{c}
Control	59.3^{g}	102.3h	120.6g	2.0^{b}	2.7^{ab}	3.0^{c}
C.V%	**2.6**	**1.1**	**1.7**	**16.5**	**12.6**	**6.1**

*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

1.2 Effect of local mycorrhizal isolated and phosphorus fertilizers on top fresh and dry weight:

The results indicated a significant increase in top fresh weight. The highest value of top fresh weight was reported with date palm mycorrhizal inoculums both treatments (MD1 and MD2), followed by sugarcane mycorrhizal inoculums. It is apparent from these findings that the mycorrhiza isolated from date palm is superior in improving the measured top fresh weight. The statistical analysis also showed that the same results among top dry weight. These results in are agreement with the findings of the research conducted by Mahdi (1993) and Mohamed *et. al*. (2008).

Table (3). Effect of the treatments used on *Zea maize* top fresh and dry weight at different sampling intervals.

Treatments	Top fresh weight g/plant			Top dry weight g/plant		
	(1) Month	(2) Month	(3) Month	(1) Month	(2) Month	(3) Month
MS1	72.3^{d}	111.0d^{e}	160.6^{de}	62.0^{d}	96.3d^{de}	143.6^{e}
MS2	78.6^{c}	133.0^{c}	183.3^{c}	68.0^{c}	117.3^{c}	172.3^{c}
MA1	60.6^{e}	120.3^{cd}	159.6^{e}	49.0^{e}	108.6^{cd}	145.0^{e}
MA2	71.0^{d}	126.3^{cd}	168.6^{d}	58.0^{d}	115.0^{c}	157.6^{d}
MD1	97.3^{b}	180.0^{b}	258.6^{b}	82.3^{b}	168.3^{b}	246.0^{b}
MD2	108.6^{a}	281.0^{a}	362.6^{a}	96.6^{a}	267.3^{a}	350.3^{a}
P	54.3^{f}	119.0^{cde}	132.0^{g}	42.0^{f}	103.0^{cde}	119.3^{f}
P (2)	57.3^{ef}	129.0^{c}	150.0^{f}	46.0^{ef}	116.6^{c}	138.6^{e}
Control	32.6^{g}	104.0^{e}	122.3^{h}	22.6^{g}	91.0^{e}	112.0^{f}
C.V%	**4.8**	**6.3**	**2.4**	**4.8**	**7.4**	**3.1**

*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

1.3 Effect of local mycorrhizal isolates and phosphorus fertilizers on root fresh and dry weight:

The root fresh weight was significantly increased by VA mycorrhiza. The best values were observed in date palm mycorrhizal inoculums followed by sugarcane mycorrhizal inoculum. After one month from sowing, zea maize plants inoculated with mycorrhiza isolated from date palm significantly increased root dry weight of zea maize plants compared to plants inoculated with alfalfa and sugarcane mycorhiza. all treatments of mycorrhizal inoculums increased root dry weight when compared to the control treatment and the one to which mineral phosphorus was added at the recommended dose and over dose. The same trend was almost observed at two month and harvest time (three month from sowing). Similar results were reported by Mahdi (1993) and Mohamed *et al*. (2008).

Table (4). Effect of the treatments used on *Zea maize* root fresh and dry weight at different sampling intervals.

Treatments	Root fresh weight g/plant			Root dry weight g/plant		
	(1) Month	(2) Month	(3) Month	(1) Month	(2) Month	(3) Month
MS1	6.3^{def}	16.0^{cd}	23.3^{cd}	4.0^{def}	7.6^{cd}	13.0^{def}
MS2	10.0^{cd}	19.0^{c}	27.0^{c}	5.6bc	9.6^{cd}	18.0^{c}
MA1	9.0^{cde}	14.0^{d}	20.3^{de}	3.3^{c}	5.6^{cd}	9.6^{fg}
MA2	11.0^{c}	14.6^{cd}	24.6^{cd}	7.6b	11.6^{c}	15.0^{cd}
MD1	20.6^{b}	28.0^{b}	36.3^{b}	16.3a	21.6^{b}	24.0^{b}
MD2	29.6^{a}	37.0^{a}	45.0^{a}	18.6^{a}	28.3^{a}	32.0^{a}
P	6.6^{def}	14.0^{d}	26.3^{c}	3.6^{c}	8.3^{cd}	14.6^{cde}
P (2)	9.3^{cde}	19.0^{c}	28.3^{c}	5.6^{bc}	9.6^{cd}	16.0^{cd}
Control	5.6^{ef}	11.3^{d}	16.6^{e}	3.0^{c}	5.0^{d}	7.0^{g}
C.V%	**19.9**	**14.9**	**12.1**	**28.8**	**29.2**	**13.4**

*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

1.4 Effect of local mycorrhizal isolates on *Zea maize* root colonization and spore density.

Treatments inoculated with local mycorrhiza isolated from date palm, alfalfa and sugarcane significantly improved root colonization, also observed treatment of (MD2) superior compare to other treatments. Treatments inoculated with local mycorrhizal isolated from date palm, alfalfa and sugarcane significantly improved spore density, also observed treatment of mycorrhizal isolate from date palm (MD2) superior compare to other treatment.

Table (5). Effect of the treatments used on *Zea maize* root colonization and spore density at different sampling intervals.

Treatments	Root colonization (%)			Spore density		
	(1) Month	(2) Month	(3) Month	(1) Month	(2) Month	(3) Month
MS1	28.3^{f}	40.6^{f}	51.0^{f}	436.7^{e}	543.3^{f}	583.3^{c}
MS2	32.6^{e}	45.6^{e}	55.3^{e}	727.0^{d}	770.0^{d}	780.0^{bc}
MA1	38.6^{d}	50.0^{d}	64.6^{d}	733.7^{d}	786.0^{d}	808.3^{bc}
MA2	46.6^{c}	53.0^{c}	73.3^{c}	830.3^{c}	885.3^{c}	983.7^{b}
MD1	58.6^{b}	64.0^{b}	80.0^{b}	866.7^{b}	947.7^{b}	1017.0^{b}
MD2	63.3^{a}	69.3^{a}	84.3^{a}	962.7^{a}	1030.7^{a}	1393.7^{a}
P	7.6^{g}	7.3^{h}	21.6^{f}	82.0^{f}	95.3^{f}	107.3^{d}
P (2)	7.3^{g}	9.3^{g}	28.6^{g}	74.0^{f}	87.0^{h}	104.7^{d}
Control	9.0^{g}	11.0^{g}	28.0^{g}	96.0^{f}	112.7^{f}	140.3^{d}
C.V%	**3.7**	**3.3**	**3.4**	**2.9**	**2.7**	**26.0**

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

1.5 Effect of local mycorrhiza isolated and phosphorus fertilization on *Zea maize* plant content of N, P, K.

Treatments of mycorrhizal ioculums enhanced plant total nitrogen uptake followed by phosphorus fertilization compared with controls. However, the highest value after harvest recorded both by mycorrhizal (MD2) and (MA2) Compared with other treatments even controls. Treatments of mycorrhizal ioculums enhanced plant phosphorus uptake followed by phosphorus fertilizer dose (2) compared with controls. However, the highest value after harvest recorded by mycorrhizal (MD2), followed by (P2) Compared with other treatments even controls. However, the highest value of K tissue content after harvest recorded by mycorrhizal (MS2), (MD1) and (MD2) followed by (MS1) and (MA2) Compared with other treatments even controls. These results agreement with the findings of the research conducted by (Artursson *et. al*., 2006), Govindarajulu *et. al*., (2005), Harrier, *et. al*., (2001), Bressan et. al., (2001), Liu *et. al*., (2002), Hayman (1983), Plenchette *e.t al*., (1983), Van der Heijden et. al., (1998) and Hodge *et. al*., (2001), Galal (1993) and Mahdi *et. al*., (2004).

Table (6). Effect of local mycorrhiza isolated and phosphorus fertilization on tissue *zea mays* (N, P, K) (%) at different sampling intervals.

Treatments	Nitrogen(N)			Phosphorus (P)			Potassium(K)		
	(1) Month	(2) Month	(3) Month	(1) Month	(2) Month	(3) Month	(1) Month	(2) Month	(3) Month
MS(1)	1.5^{f}	1.7^{cd}	2.8^{d}	0.00001^{f}	0.00001^{h}	0.0002^{f}	0.04^{d}	0.06^{b}	0.07^{ab}
MS(2)	1.6^{e}	2.0^{c}	2.9^{c}	0.00002^{e}	0.00002^{g}	0.0004^{e}	0.06^{c}	0.09^{a}	0.09^{a}
MA(1)	1.8^{d}	2.8^{b}	3.0^{bc}	0.00003^{de}	0.00003^{f}	0.0004^{e}	0.07^{b}	0.05^{bc}	0.04^{c}
MA(2)	2.0^{c}	3.0^{ab}	3.4^{a}	0.00003^{de}	0.00004^{e}	0.0005^{d}	0.07^{b}	0.07^{ab}	0.07^{ab}
MD(1)	2.1^{b}	3.0^{ab}	3.3^{ab}	0.00007^{b}	0.00007^{b}	0.0008^{b}	0.08^{a}	0.08^{a}	0.08^{a}
MD(2)	2.3^{a}	3.2^{a}	3.5^{a}	0.00009^{a}	0.00009^{a}	0.0009^{a}	0.08^{a}	0.08^{a}	0.08^{a}
P	1.1^{g}	1.6^{d}	2.2^{f}	0.00003^{de}	0.00005^{d}	0.0006^{c}	0.03^{e}	0.03^{cd}	0.04^{c}
P (2)	1.2^{g}	2.0^{c}	2.5^{e}	0.00005^{c}	0.00006^{c}	0.0008^{b}	0.03^{e}	0.03^{cd}	0.04^{c}
Control	1.0^{h}	1.6^{d}	2.3^{f}	0.00001^{f}	0.00001^{h}	0.0001^{g}	0.01^{f}	0.01^{d}	0.01^{d}
C.V%	**2.6**	**6.0**	**2.8**	**12.6**	**7.4**	**8.0**	**7.4**	**22.0**	**22.9**

*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.

2. Groundnuts:

2.1 Effect of treatments on plant height and color rating:

The data are presented in table (4.16). Appendix (D1- D2). After one month from sowing, the highest plant height, which could be attributed to mycorrhizal positive effect was observed in peanut plants inoculated with mixture mycorrhiza dose (1), and urea treatment. The lowest height, however, was observed in the treatment of control. However, after two month from sowing, the plants inoculated with mycorrhiza dose (2) was superior compared to all other treatments and significantly exceeded all other treatments including even the plants inoculated with mixture mycorrhiza dose (1). After three month from sowing the plant inoculated with mixture mycorrhizal dose (2) record highest plant height followed by plant inoculated with mycorrhizal dose (1). These results agreement with the findings of the research conducted by Mahdi (1993). But also observed the treatment of alfalfa mycorrhizal was not significant results compared to other treatments. The treatments of urea and both *Rhizobium* strains (INRRI 24 and ICRISAT 7001) significantly improved peanut plant color rating, also were no significant differences. All these results referring to mycorrhize infection, however, both doses of mycorrhize superior in all paremeters.

Table (7). Effect of the treatments used on groundnuts plant height and color rating at different sampling intervals

Treatments	Plant height cm/plant			Color rating		
	(1) Month	(2) Month	(3) Month	(1) Month	(2) Month	(3) Month
M.Mixture (1)	9.0^{a}	20.3^{b}	20.6^{b}	2.0^{c}	2.0^{c}	2.0^{c}
M.Mixture (2)	8.3^{b}	22.3^{a}	22.6^{a}	2.0^{c}	2.6^{bc}	2.6^{bc}
INNRI 24	8.0^{ab}	18.3^{c}	18.6^{c}	2.6^{ab}	3.0^{ab}	3.6^{a}
ICRISAT 7001	7.6^{ab}	16.6^{d}	$17.3d^{c}$	3.0^{a}	3.6^{a}	3.6^{a}
N	9.0^{a}	16.6^{d}	18.6^{c}	3.0^{a}	3.6^{a}	4.0^{a}
Amm.SO_4	7.3^{bc}	17.0^{cd}	17.6^{dc}	2.3^{bc}	$3.0a^{b}$	3.3^{ab}
P	7.3^{bc}	14.3^{e}	16.0^{de}	2.0^{c}	2.3^{bc}	2.0^{c}
M. Alfalfa	8.0^{ab}	16.6^{d}	17.0^{dc}	2.0^{c}	3.0^{ab}	2.6^{bc}
Control	6.0^{c}	12.0^{f}	14.3^{e}	2.0^{c}	2.3^{bc}	2.0^{c}
C.V%	**10.0**	**5.1**	**5.7**	**12.3**	**18.3**	**18.2**

*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

2.2 Effect of treatments on top fresh weight and top dry weight:

The data are presented in table (4.17). Appendix (D3- D4). The statistical analysis showed that plant inoculated with *Rhizobium* (INRRI 24) recorded superior top fresh weight in the three month after sowing respectively compared with control. However, plants inoculated with mixture mycorrhizal dose (2) recorded (26.6gm), (31.8gm) respectively considered as the highest result after treatment of *Rhizobium* (INRRI 24) in the two and three months after sowing. Also the reults showed that plants inoculated with *Rhizobium* (INRRI 24) record superior top dry weight in the three Months after sowing respectively compared with control. Also the treatment of recommended dose of superphosphate recorded lowest value in one month from sowing. These results could be attributed to mycorrhizae infection enhanced N and P uptake. These results agreement with the findings of the research conducted by Galal (1993) and Atabani (1988).

Table (8). Effect of treatments on groundnuts top fresh and dry weight at different sampling intervals

Treatments	Top fresh weight g/plant			Top dry weight g/plant		
	(1) Month	(2) Month	(3) Month	(1) Month	(2) Month	(3) Month
M.Mixture (1)	10.3^{d}	17.1^{d}	23.0^{d}	5.1^{d}	10.9^{c}	16.6^{b}
M.Mixture (2)	13.0^{c}	26.6^{b}	31.8^{b}	6.9^{c}	13.8^{b}	14.0^{c}
INNRI 24	23.3^{a}	45.6^{a}	56.3^{a}	12.1^{a}	26.6^{a}	28.6^{a}
ICRISAT 7001	16.3^{b}	23.0^{c}	26.6^{c}	8.6^{b}	10.7^{c}	15.3^{bc}
N	7.3^{ef}	12.9^{ef}	17.3^{e}	$4.3d^{e}$	7.2^{d}	12.6^{d}
Amm.SO_4	8.0^{e}	14.4^{de}	18.3^{e}	3.5^{ef}	7.3^{d}	11.3^{de}
P	6.3^{ef}	12.6^{ef}	18.6^{e}	2.8^{f}	6.4^{de}	10.0^{ef}
M. Alfalfa	7.3^{ef}	10.9^{fg}	16.6^{e}	3.7^{ef}	5.9^{de}	8.6^{fg}
Control	5.6^{f}	8.3^{g}	11.6^{f}	3.3^{ef}	4.7^{e}	7.3^{g}
C.V%	**9.0**	**8.2**	**7.3**	**12.3**	**13.2**	**7.9**

*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

2.3 Effect of treatments on root fresh and dry weight:

The data are presented in table (4.18). Appendix (D5- D6). Treatments inoculated with *Rhizobium* both (INRRI 24 and ICRISAT 7001) significantly improved root fresh weights, followed by treatment of mineral phosphorus recommended dose compared to the uninoculated controls, in three Month respectively. Treatments inoculated with *Rhizobium* (both INRRI 24 and ICRISAT 7001) significantly improved root dry weights, followed by treatment of mineral phosphorus recommended dose compared to the uninoculated controls. These results are agreement with Ahmed *et al*., (2009) and Tian *et al*. (2002).

Table (9). Effect of the treatments used on groundnuts root fresh and dry weight at different sampling intervals (g/plant).

Treatments	Root fresh weight			Root dry weight		
	(1) Month	(2) Month	(3) Month	(1) Month	(2) Month	(3) Month
M.Mixture (1)	0.7^{ef}	1.4^{c}	1.6^{c}	0.6^{c}	1.0^{d}	1.2^{c}
M.Mixture (2)	0.8^{cd}	1.6^{bc}	1.6^{c}	0.5^{cd}	1.2^{c}	1.2^{c}
INNRI 24	1.4^{a}	2.3^{a}	2.6^{a}	0.9^{a}	1.9^{a}	2.2^{a}
ICRISAT 7001	1.1b	1.8^{b}	2.0^{b}	0.7^{b}	1.5^{b}	1.5^{b}
N	0.7^{ef}	0.9^{de}	1.1^{d}	0.4^{cd}	0.6^{e}	0.9^{d}
Amm.SO4	0.6^{g}	0.7^{e}	1.0^{d}	0.4^{cd}	0.4^{f}	0.7^{d}
P	0.9^{c}	1.6^{bc}	1.9^{b}	0.7^{b}	1.2^{c}	1.6^{b}
M. Alfalfa	0.8^{cd}	1.1^{d}	1.2^{d}	0.5^{cd}	0.8^{e}	0.9^{d}
Control	0.3^{h}	0.6^{e}	1.0^{d}	0.1^{e}	0.3^{f}	0.7^{d}
C.V%	6.6	10.7	7.7	11.2	10.1	9.9

*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

2.4 The Performance of *Rhizobium* strains with the tested grounnuts cultivar in nodulation.

The data are presented in table (4.19-4.20). Appendix (D7- D10). Results showed that the local of *Rhizobium* **(**INRRI 24) significantly improved both number and mass of nodulation both main and lateral roots compared with alien *Rhizobium* **(**ICRISAT 7001) in the three Month, but also observed there was no significant differences between both treatment in mass of nodules in the three month. These results agreement with the findings of the research conducted by Abdelgani *e.t al*., (2003).

Table (10). Effect of *Rhizobium* strains on groundnuts number and mass main roots of nodules at different sampling intervals

Treatments	(1) Month		(2) Month		(3) Month	
	Number/ plant	Mass mg/plant	Number/ plant	Mass mg/plant	Number/ plant	Mass mg/plant
M.Mixture (1)	20.3^{cd}	13.6^{c}	13.3^{cd}	4.0^{d}	$4.3d^{e}$	2.0^{b}
M.Mixture (2)	21.3^{c}	13.6^{c}	16.6^{c}	3.6^{d}	$5.0d^{e}$	3.0^{b}
INNRI 24	96.3^{a}	85.3^{a}	93.0^{a}	77.0^{a}	54.6^{a}	36.0^{a}
ICRISAT 7001	75.0^{b}	68.3^{b}	67.0^{b}	57.0^{b}	42.3^{b}	32.6^{a}
N	13.3^{e}	10.3^{cd}	$12.3d^{e}$	13.0^{c}	7.3^{cd}	5.3^{b}
Amm.SO_4	$15.3d^{e}$	10.3^{cd}	13.0^{de}	13.3^{c}	8.6^{c}	7.0^{b}
P	11.0^{e}	7.0^{d}	9.6^{e}	8.6^{cd}	$6.3cd^{e}$	4.6^{b}
M. Alfalfa	13.3^{e}	7.6^{d}	4.3^{f}	2.6^{d}	3.6^{e}	2.6^{b}
Control	13.6^{e}	8.0^{d}	5.6^{f}	5.0^{d}	4.0^{e}	2.0^{b}
C.V%	**10.2**	**10.7**	**7.4**	**17.7**	**13.6**	**30.7**

*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 (11). Effect of *Rhizobium* strains on groundnuts in number and mass of nodules lateral roots at different sampling intervals.

Treatments	(1) Month		(2) Month		(3) Month	
	Number/ plant	Mass/mg	Number/ plant	Mass/mg	Number/ plant	Mass/mg
M.Mixture (1)	10.0^{bc}	4.6^{bc}	3.6^{bc}	3.3^{bc}	3.6^{c}	2.0^{bc}
M.Mixture (2)	7.0^{cd}	4.3^{bc}	4.6^{bc}	3.6^{bc}	4.3^{c}	2.0^{bc}
INNRI 24	34.0^{a}	28.3^{a}	23.6^{a}	18.6^{a}	17.6^{a}	9.0^{a}
ICRISAT 7001	29.6^{a}	26.0^{a}	23.0^{a}	19.0^{a}	14.0^{b}	7.6^{a}
N	13.3^{b}	8.6^{b}	8.3^{b}	5.6^{b}	4.0^{c}	2.3^{b}
Amm.SO_4	7.6^{cd}	6.0^{bc}	4.3^{bc}	3.3^{bc}	3.3^{c}	1.3^{bc}
P	8.3^{bc}	5.6^{bc}	4.6^{bc}	3.3^{bc}	4.0^{c}	2.0^{bc}
M. Alfalfa	5.0^{cd}	2.6^{c}	2.0^{c}	1.3^{c}	1.6^{c}	1.0^{bc}
Control	2.6^{d}	2.6^{c}	1.3^{c}	1.3^{bc}	1.0^{c}	0.6^{c}
C.V%	**24.4**	**32.5**	**35.2**	**27.8**	**33.1**	**27.8**

*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

2.5 Effect of local mycorrhizal on groundnuts root colonization and spore density.

The data are presented in table (4.21). Appendix (D11- D12). Treatments inoculated with local mycorrhiza isolates (M.mixture (1), M.mixture (2) and M.alfalfa) significantly improved root colonization, also observed treatment of M.mixture (2) superior compare to other treatments. Treatments inoculated with local mycorrhizal isolated from date palm, alfalfa and sugarcane significantly improved spore density, also observed treatment of mycorrhizal mixture (M.Mixture (2)) superior compare to other treatments. High spore's density in the soil leads to an increase in root infection.

Table (12). Effect of the treatments used on groundnuts root colonization and spore density at different sampling intervals.

Treatments	Plant root colonization (%)			Spore density		
	(1) Month	(2) Month	(3) Month	(1) Month	(2) Month	(3) Month
M.Mixture (1)	48.3^{b}	68.6^{b}	72.8^{b}	700.3^{b}	847.3^{b}	1021^{b}
M.Mixture (2)	58.6^{a}	77.3^{a}	81.5^{a}	863.6^{a}	928.0^{a}	1083.3^{a}
INNRI 24	9.6^{d}	15.0^{d}	17.6^{d}	77.3^{c}	89.6^{g}	122.0^{e}
ICRISAT 7001	10.6^{d}	13.6^{d}	16.3^{de}	60.0^{c}	67.0^{f}	80.0f
N	8.0^{d}	8.3^{ef}	11.0^{f}	66.3^{c}	114.0^{e}	137.6^{e}
Amm.SO4	8.3^{d}	8.3^{ef}	11.0^{f}	59.0^{c}	111.0^{e}	113.0^{ef}
P	8.0^{d}	10.0^{e}	14.6^{e}	73.3^{c}	103.0^{e}	116.0^{ef}
M. Alfalfa	36.3^{c}	40.6^{c}	54.0^{c}	689.0^{b}	799.6^{c}	891.3^{c}
Control	4.0^{e}	7.0^{f}	11.6^{f}	91.0c	149.0^{d}	187.3^{d}
C.V%	**8.3**	**6.0**	**4.9**	**5.5**	**1.5**	**1.9**

*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

2.6 Effect of local mycorrhiza isolates, *Rhizobium* strains and fertilization on groundnuts plant content of N, P and K.

Table (4.22) and appendix (D13 – D15) present the data collected. After one month and two Months from sowing, treatments both (urea recommended dose and ammonium sulphate recommended dose) enhanced plant total nitrogen uptake followed by *Rhizobium* strains both (INRRI 24 and ICRISAT 7001) compared with controls. However, the highest value after three months recorded by both *Rhizobium* (INRRI 24) and urea followed by both treatments (ammonium sulphate recommended dose) and *Rhizobium* strain (ICRISAT 7001). Compared with other treatments even controls. After month and two Month from sowing, treatments both (superphosphate recommended dose and mycorrhizal inoculm dose (2)) enhanced peanut plant phosphorus uptake followed by other treatments. The highest value after harvest recorded by mycorrhizal inoculm dose (2) followed by mycorrhizal inoculm dose (1), compared with other treatment even controls. After month, two months and three months from sowing, treatment mycorrhizal inoculm dose (2) enhanced plant potassium uptake compared with controls. These results can be referring to biological nitrogen fixation and

biological fertilizers. These results agreement with the findings of the research conducted by Bressan *et. al*., (2001), Liu *et. al*., (2002), Maremmani (2003) and Artursson *et. al*.,(2006).

Table (13). Effect of local mycorrhiza isolates, *Rhizobium* strains and fertilization on groundnuts N, P, K (%).

Treatments	Sampling intervals (N)			Sampling intervals (P)			Sampling intervals (K)		
	(1) Month	(2) Month	(3) Month	(1) Month	(2) Month	(3) Month	(1) Month	(2) Month	(3) Month
M.Mixture (1)	1.2^{ef}	1.7^{d}	3.1^{c}	0.00004^{bc}	0.0002^{bc}	0.0003^{b}	0.05^{b}	0.05^{b}	0.07^{a}
M.Mixture (2)	1.4^{d}	2.0^{d}	3.3^{bc}	0.00006^{a}	0.00023^{ab}	0.0005^{a}	0.08^{a}	0.09^{a}	0.09^{a}
INNRI 24	2.1^{b}	3.0^{b}	4.0^{a}	0.00003^{cd}	0.00013^{cd}	0.0002^{cd}	0.003^{c}	0.004^{c}	0.004^{c}
ICRISAT 7001	1.9^{c}	2.6^{c}	3.4^{b}	0.00001^{f}	0.00013^{cd}	0.0002^{cd}	0.003^{c}	0.004^{c}	0.004^{c}
N	2.6^{a}	3.3^{a}	3.8^{a}	0.00002^{ef}	0.0001^{de}	0.0001^{d}	0.00^{1}	0.002^{c}	0.002^{c}
$Amm.SO_4$	2.5^{a}	3.4^{a}	3.4^{b}	0.00002^{ef}	0.00013^{cd}	0.0001^{d}	0.001^{c}	0.002^{c}	0.002^{c}
P	1.1^{f}	1.6^{de}	2.1^{e}	0.00005^{a}	0.0003^{a}	0.0003^{b}	0.002^{c}	0.003^{c}	0.004^{c}
M. Alfalfa	1.3^{de}	1.4^{ef}	$2.^{7d}$	0.00003^{cd}	0.0002^{bc}	0.0002^{bc}	0.05^{b}	0.06^{b}	0.02^{b}
Control	0.9^{g}	1.1^{f}	1.5^{f}	0.00002^{ef}	0.00004^{e}	0.0001^{d}	0.001^{c}	0.001^{c}	0.001^{c}
C.V%	**5.0**	**8.5**	**5.5**	**24.2**	**24.5**	**20.3**	**20.8**	**20.8**	**54.0**

*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.

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