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Effect of Mycorrhiza Strains Isolated from AL-Jabal AL-Akhdar Forests Application on the Vegetative Growth of Maize (Zea Mays L.) Plant under (Sirte Saline Soil-Libya)

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ABSTRACT

In this study, a salt-stress pot experiment was conducted to evaluate the effects of AMF on growth of Zea mays L. plants, grown in sirt saline soil-Libya, which was reflected on fertilization of soil and reduction of both cost and environ mental pollution. Experimental units were supplied with chemical fertilizers as recommended to Zea mays L. plants, with the exception of phorphorus being added with three concentrations (0, 50 and 100 kg p/ha). Results showed the a significant increase of the strain Rhizophagus intraradices where the infection rate was 92%, followed by the strain which was 90.33% and the lowest infection rate was for the strain Scutellospora sp., which was 83%. Also showed that the fungi Scutellospora sp., Claroideoglomus etunicatum were significantly better strains tested on wet and dry weight of shoot. Also the interference between inoculation and levels of phosphate fertilization in the saline soils the studied characters significantly, (mean weight diameter, percentage of root infection, root length, dry weights of shoot and root growth. The interference between inoculation and Levels of phosphorus in the saline soils, increased the studied characters significantly, in particular, significant increase for most of treatments of the mycorrhizal strains. Where it was observed that effect differed in the studied trait and the difference of the strain with difference of strain each trait.

Keywords: *Mycorrhizal strain, Growth, Maize, Slain soils, Inoculation.*

1. Introduction

The use of Arbuscular Mycorrhizal Fungi (AMF) is considered one of the effective organic ways to increase the crops productivity [1]. The effects of different arbuscular mycorrhizal fungi (AMF) have been evaluated under field conditions for cucumber, ***Glomus mosseae*** and ***Glomus etunicatum*** inoculated cucumber. The field experiment results showed that mycorrhiza inoculation significantly increased cucumber seedling survival, fruit yield, P and Zn shoot concentrations [2]. This is particularly the case in regions with high rates of evaporation, like arid and semi-arid areas [3]. AMF treatments increased plant growth performance, dependent on AMF species [4]. The negative intensified effects of salinity in the arid and semi-arid lands were due to the heavy fertilization and improper irrigation [5]. The AM fungus positively influenced maize growth and nutrient uptake [6]. The term “mycorrhiza” was coined to describe the distinctive properties of the rhizosphere surrounding and influenced by mycorrhizas [7]. AM fungi are capable of increasing the endurance of plants to salt stress by enhancing plant nutrient uptake and ion balance [8]. AM fungi alleviate salt stress in plant and have a capacity to protect soil enzymes and soil organic matter [9]. AM may improve osmotic regular via accumulation of soluble sugars and electrolyte concentrations in root cells [10]. Maize is classified as a salt-sensitive plant [11].in many previous studies, it was found that inoculation with AMF can alleviate salt stress [12]. Improved salt tolerance following mycorrhizal colonization may be the result of a more efficient nutrient uptake [13], ion balance [14], protection of enzyme activities [15], increase in photosynthesis ability [16] and facilitation of water uptake in plants [17].

Objective: The aim of this study was to effect of mycorrhiza strains isolated from AL-Jabal AL-Akhdar forests application on the vegetative growth of Maize (Zea mays l.) plant under (Sirte saline soil–Libya).

2. Materials and Methods

1. Study site: A Libyan city located in the north on overlooking the Mediterranean Sea. We will select a particular piece of land that represents the area of the study. 2. The pot experiment was conducted during the period extended from 1 Mar. to 30 May., 2020 at the greenhouse of the Institute of Bioscience (IBS), Omar Al-Mukhtar University, Al-Bayda, Libya to examine the effect of inoculation with seven AMF strains: *Rhizophagus intraradices* (MF599223), *Claroideoglomus etunicatum* (MF599211), *Gigspora sp* (MF5992 22), *Entrophos Porainfeqeas* (MF 599211), *Scutellospora sp*., (MF599226), *Archaeospora trappei* (MF5992 13), *funneliformis mosseae* (mf599221), previously isolated from the (AL-Jabal AL-Akhdar forests -Libya) [18]. 3. Application of endomycorrhizae: A commercially and A cultivar of Zea mays L., that belongs to the Graminae family (Department of Horticulture, Faculty of Agriculture, University of Omar AL Mukhtar). Three maize seeds were surface-sterilized, planted in a 10 kg pot (40 x 40 cm) and inoculated with single-strain AFM inocula: addition fungal species of mycorrhizae identified as seedbed, as biofertilizer consisting of fungus spores, infected roots and soil resulting from propagation, treatment Mycorrhizae: M0: Control, ***Rhizophagus intraradices*** (MF599223), ***Claroideoglomus etunicatum*** (MF599211), ***Gigspora sp*** (MF599222), ***Entrophos Porainfeqeas*** (MF 599211), ***Scutellospora sp.,*** (MF599226), ***Archaeospora trappei*** (MF599213), ***funneliformis mosseae*** (AM 599221).

Addition of three phosphate fertilization levels: with out of phosphate fertilizer, addition of super phosphate (F0=without adding phosphorus), (F1=Add super phosphate Triplex at 50 g/h,) (F2=add of super phosphate at aerate of 100 kg/ha).

- ***Harvesting: Zea mays L., after transplanting***

- ***Spore Recovery***

Mycorrhizae spores were extracted from 100-gram rhizosphere soil samples by wet sieving and decanting technique [19].

- Observation parameter: The parameter observed in this experiment was (1) leave area cm^2/plant by the plain meter. Shoot weight wet g/plant. Shoot weight dry g/plant. (6) Root weight wet g/plant. (7) Root weight dry g/plant.

Soil Analysis: The physico-chemical properties of the soil such as texture, pH, EC, available nitrogen and available phosphorus [20].

Table 1. Physicochemical characteristics of soil in the study location

Physical properties	Value
Bulk density g/cm^3	1.64
Field capacity %	18.5
Soil texture	sandy

Clay %	8
Silt %	15
Sand %	77
Moisture content %	1.46

Chemical Properties		**Value**
PH		8.4
Electrical connection dS m^{-1}		6.58
Dissolved ions meq\l	**Ca^{+}**	20
	Mg^{+2}	25
	K^{+}	10
	Cl^{-}	4.75
	Co_3^{-2}	0
Cation Exchange Capacity		21.4
Calcium Carbonate%		3.75
Available Phosphorous Mg/L		3.14
Organic Matter %		1.58
Total Nitrogen Mg/L		34.2

Statistical analysis

Statistical analysis was performed using SPSS 19.0 statistical program performing first a one-way anova followed by the test at P<0.001 as the significance cut-off. Two independent statistical analyses: the first to analyze data from the eight AMF treatments within with the three phosphate fertilization levels the second one to analyze data from each fungal species at phosphate fertilization levels and four replicates.

3. Results

In the greenhouse experiment, 7 isolated, identified and screened for their resists salinity soil and growth promoting activity were selected as inocula strains. These strains were further examined as mycorrhizal biofertilizer. In Table No. 2 for calculating the rate of infection with Mycorrhizal fungi, the results showed the showed a significant increase of the strain ***Rhizophagus intraradices*** where the infection rate was 92%, followed by the strain which was 90.33% and the lowest infection rate was for the strain ***Scutellospora sp***.,

which was 83%. In the case of adding levels of phosphate fertilization, the percentage of infection decreased with the addition of phosphate fertilizer. At a phosphate fertilization level of 50 kg p/ha, the highest percentage of infection was with a strain *Rhizophagus* ***intraradices*** which was 65.67% and the lowest with a strain ***Archaeospora trappei*** which was 23.67%, When doubling the amount of phosphate fertilizer 100 kg p/ha, the highest infection was with strain ***Clarhoideoglomus etunicatum*** treatment and amounted to 82% and the lowest percentage with strain ***Entrophos Porainfeqeas*** treatment was 48%. Plant growth parameters including plant height, leaf area and shoot and root dry weight were negatively affected by salinity. However, mycorrhizal plants showed higher growth parameters under saline condition compared to non-inoculated salt affected plants [21].

Table 2. Effect treatment inocula with mycorrhizal strains and with the phosphate fertilization levels on Percent infection of mycorrhizal%

Mycorrhizae	**Fertilizer P as super phosphate**			**Mycorrhizae average**
	Zero kg p/ha	**50 kg p/ha**	**100 kg p/ha**	
No inocula mycorrhizal	0	0	0	**0**
Rhizophagus intraradices	92.00±3.00	65.67±6.51	82.33±6.81	**80.00±12.55**
Clarhoideoglomus etunicatum	89.67±1.53	51.33±4.16	75.33±4.73	**72.11±17.08**
Gigspora sp	86.67±1.53	41.00±2.65	64.33±1.53	**64.00±19.85**
Entrophos Porainfeqeas	87.33±2.08	33.67±3.06	48.33±8.14	**59.78±29.22**
Scutellospora sp.,	92.00±2.00	42.67±1.53	71.33±4.51	**68.67±21.61**
Archaeospora trappei	83.67±5.13	23.67±2.08	63.67±1.53	**57.00±26.61**
funneliformis mosseae	97.67±2.08	35.33±1.53	53.33±2.52	**62.11±27.84**
Fertilizer P average	**80.17±30.48**	**37.21±17.66**	**58.46±22.18**	
F-value		**For fertilizer P→ 855.42*** **For Mycorrhizae → 361.194*** **For F+M→ 35.84***		

*** Significant variation at P< 0.001

In table No.3, Results showed that the development of maize a significant increase in the fresh weight of the shoot when inoculated with mycorrhizal fungi for most of the isolates compared to the control treatment, the highest significant vale (333 g/plant) with inoculated with strain ***Scutellospora* sp** compared to the control, see reviews by (31); (32). And when phosphate fertilizer was added at a level of 50 kg phosphate/ha with fungus inoculation ***Rhizophagus intraradices*** the highest value of the vegetative weight was recorded and

was (337 g/plant)and at a level of 100 kg phosphorous/ha, the highest significant value was (340 g/plant) in the case of fungus inoculation ***Rhizophagus intraradices***. While all mycorrhizal strains treatments in the wet shoot weight were superior to the two treatments fertilized with phosphorous levels of 50 and 100 kg phosphorous/ha, except for the saline treatment with strain ***funneliformis mosseae***. The addition of phosphate fertilizer 100 kg/ha increased the weight of the wet shoot by 5 g/plant and the increase was insignificant.

Table 3. Effect treatment inocula with mycorrhizal strains and with the phosphate fertilization levels on Shoot wet weight g/plant

Mycorrhizae	**Fertilizer P as super phosphate**			**Mycorrhizae average**
	Zero kg p/ha	**50 kg p/ha**	**100 kg p/ha**	
No inocula mycorrhizal	164.00±12.12	259.00±6.56	293.33±6.66	**238.78±58.52**
Rhizophagus intraradices	330.00±13.23	337.67±11.59	340.67±8.96	**336.11±10.96**
Claroideoglomus etunicatum	314.67±5.51	236.67±10.60	263.00±10.82	**271.44±35.30**
Gigspora sp	291.00±11.36	251.67±3.79	315.67±3.79	**286.11±28.65**
Entrophos Porainfeqeas	303.67±6.81	225.00±8.89	295.67±23.29	**274.78±39.66**
Scutellospora sp.,	333.67±10.26	262.33±29.48	300.00±5.29	**298.67±34.72**
Archaeospora trappei	288.67±11.02	321.00±8.66	276.67±4.73	**295.44±21.19**
funneliformis mosseae	284.33±6.66	221.67±3.21	267.67±4.51	**257.89±28.44**
Fertilizer P Average	**288.75±52.00**	**264.38±42.44**	**294.08±26.10**	
F-value	**For fertilizer P→ 48.08*** **For Mycorrhizae → 61.76*** **For F+M→ 33.73***			

*** Significant variation at P<0.001

In table No.4, However, AMF application significantly enhanced dry weight of the shoot parameters (P<0.001) compared with the non-mycorrhizal plants under salt stress. It was clear from the results that a significant increase in the dry weight of the shoot occurred when inoculating with the tested different strains of Mycorrhizae compared with the control treatment. Where the highest value of dry weight of the shoot total was in the treatment of a strain ***Claroideoglomus etunicatum***, its value was 134.8 g/plant. And when adding phosphate fertilization, phosphate fertilization gave 50 kg/ha less than all treatments inoculated together without phosphate fertilization, when adding fertilizer 50 kg p/ha with inoculation with a strain ***Rhizophagus intraradices*** the highest value of the dry shoot total was 122 g/plant, and the lowest value in the of interaction was when inoculating with a strain ***Scutellospora sp***., It was 93g/ plant ***funneliformis mosseae.***

However, in the case adding phosphate fertilizer at a rate of 100 kg p/ha, it gave an increase in the dry weight of the shoot total less than some treatments of mycorrhizal strains, and it was almost similar to the treatment of inoculated with the strain ***Entrophos Porainfeqeas, Scutellospora sp.*** In the case of fertilization at a rate of 100 kg p/ha with inoculation with different strains, the highest value of the dry weight of the shoot was 126 g/plant, and the lowest value of the dry weight of the shoot was 103g/plant at the treatment of strain.

Table 4. Effect treatment inocula with mycorrhizal strains and with the phosphate fertilization levels on shoot dry weight g/plant

Mycorrhizae	**Fertilizer P**			**Mycorrhizae average**
	Zero kg p/ ha	**50 kg p/ha**	**100 kg p/ ha**	
No inocula mycorrhizal	68.33±5.51	82.00±2.65	118.33±7.64	**89.56±22.91**
Rhizophagus intraradices	123.67±5.13	122.33±4.04	106.00±4.58	**117.33±9.41**
Claroideoglomus etunicatum	134.67±10.50	119.00±1.00	106.67±5.86	**120.11±13.57**
Gigspora sp	88.00±9.54	112.33±2.52	108.00±2.00	**102.78±12.32**
Entrophos Porainfeqeas	106.00±4.58	102.67±3.51	126.00±5.00	**111.56±11.58**
Scutellospora sp.,	127.00±2.00	93.00±2.00	126.00±2.65	**115.33±16.87**
Archaeospora trappei	110.33±4.93	108.33±2.08	107.67±5.77	**108.78±4.12**
funneliformis mosseae	97.67±1.53	99.33±1.53	103.00±2.00	**100.00±2.78**
Fertilizer P average	**106.96±21.68**	**104.88±13.06**	**112.71±9.78**	
F-value	**For fertilizer P→ 17.23*** **For Mycorrhizae → 41.04*** **For F+M→ 28.81***			

*** Significant variation at P<0.001

Effect of the treatments Mycorrhizal strains with phosphate fertilization treatments on the wet weight of the roots, treatments of inoculation with Mycorrhizal strains with no addition of phosphate fertilization levels. It was observed that there was a significant increase for all treatments compared to control. The highest value of the wet weight of the root was 323 g/plant, when inoculated with the strain the lowest value of wet weight of the root with the coefficients of the Mycorrhizal strains when inoculated with the strain ***funneliformis mosseae***, it was 303 g/plant, lowest vale of wet weight of the root with coefficients of the strain ***Claroideoglomus etunicatum***, it was 164 g/plant (Table 5).

In table **No.5**, When adding a level of phosphate fertilization 50 kg/ha, the mycorrhizal fungi were not inoculated, the lowest value of the wet weight of the root was recorded, in case the treatment of the strain ***funneliformis mosseae*** the highest value of the interaction was 303 g/ plant and the lowest value in the case of the interaction in the treatment of the strain ***Claroideoglomus etunicatum*** **was** 126 g/plant. While when adding 100 kg p/ha of phosphate fertilizer level, it was without inoculation, it was insignificant and the increase was less than all the mycorrhizal treatments, in case the treatment of the strain ***Scutellospora sp.,*** it was 297 g/ plant and the minimum value of the wet weight of the root in the case the treatment of the strain ***Rhizophagus intraradices*** of was 146.7g/ plant.

Table 5. Effect treatment inocula with mycorrhizal strains and with the phosphate fertilization levels on wet root weight g/plant

Mycorrhizae	**Fertilizer P**			**Mycorrhizae average**
	Zero kg p/ ha	**50 kg p/ha**	**100 kg p/ ha**	
No inocula mycorrhizal	78.67±7.64	91.33±8.62	114.00±11.53	**94.67±17.51**
Rhizophagus intraradices	180.00±6.24	144.00±5.29	147.67±4.04	**157.22±17.75**
Claroideoglomus etunicatum	164.00±7.21	126.67±4.16	185.33±10.02	**158.67±26.53**
Gigspora sp	187.33±3.06	152.33±7.77	201.67±10.41	**180.44±22.97**
Entrophos Porainfeqeas	202.00±9.64	144.67±5.03	152.00±7.55	**166.22±27.82**
Scutellospora sp.,	248.67±25.79	178.67±9.81	297.67±6.66	**241.67±53.71**
Archaeospora trappei	281.00±8.19	284.33±6.03	254.67±9.50	**273.33±15.70**
funneliformis mosseae	323.33±11.55	303.00±5.57	285.67±12.34	**304.00±18.59**
Fertilizer P average	**208.13±73.07**	**178.13±72.46**	**204.83±65.41**	
F-value	**For fertilizer P→ 71.251***** **For Mycorrhizae → 479.652***** **For F+M→ 24.644*****			

*** Significant variation at P<0.001

The results showed the effect inoculation with Mycorrhizal strains gave a significant increase compared to the control at the least probability of 0.001, the strain ***funneliformis mosseae*** the highest dry weight of the root system was 113 g/plant, and the least effective strain the highest dry weight of the root system where the weight of the root it was 73 g/plant. When adding a level of phosphate fertilization 50 kgp/ha, it a non-significant increase in the dry weight of the root system. While when adding twice the level of phosphate fertilizer, which is 100 kg p/ha, a significant increase in the dry weight of the root it was 108 g/plant. When the

interaction between this level and the different strains tested, the highest dry weight of the root was recorded when treating strain ***Archaeospora trappei*** and it was 108 g/plant and the lowest dry weight when inoculating with strain ***Entrophos Porainfeqeas***, and it was 80 g/plant. Mycorrhizal plants produced more fresh and dry biomass, as a consequence of a better nutritional and hydric status as often reported in the literature [22].

Table 6. Effect treatment inocula with mycorrhizal strains and with the phosphate fertilization levels on Root dry weight g/plant

Mycorrhizae	Fertilizer P			Mycorrhizae average
	Zero kg p/ ha	50 kg p/ha	100 kg p/ ha	
No inocula mycorrhizal	62.67±4.04	65.67±4.51	108.33±6.66	**78.89±22.57**
Rhizophagus intraradices	89.67±8.08	64.67±7.51	102.67±4.04	**85.67±17.73**
Claroideoglomus etunicatum	73.00±5.29	73.00±6.93	107.33±11.93	**84.44±18.69**
Gigspora sp	83.00±6.24	64.33±2.31	102.33±7.09	**83.22±17.16**
Entrophos Porainfeqeas	100.33±2.31	74.33±3.06	80.33±7.57	**85.00±12.53**
Scutellospora sp.,	89.33±0.58	80.00±5.00	105.33±4.04	**91.56±11.56**
Archaeospora trappei	92.67±6.03	104.67±2.31	108.67±6.66	**102.00±8.57**
funneliformis mosseae	113.33±3.51	123.67±4.73	99.33±5.13	**112.11±11.27**
Fertilizer P average	**88.00±15.52**	**81.29±21.05**	**101.79±10.63**	
F-value	**For fertilizer P→ 79.383*** For Mycorrhizae → 34.191*** For F+M→ 19.120***			

*** Significant variation at P<0.001

In table **No.7**, found from leaf area, most influential mycorrhizal strains on the leaf area was strain ***Claroideoglomus etunicatum*** and gave a leaf area of 3211.8 cm^2/plant, and the least influential strains were ***Rhizophagus intraradices*** and gave a leaf area of 2310 cm^2/plant.

And when phosphate fertilizer was added, the level of fertilization of 50 kg p/ha was not significant, when inoculation with mycorrhizal strains, and the highest value of the leaf area was 35838 cm^2/plant.

Results showed a non-significant increase in the leaf area when adding a level of 100 kg p/ha, without inoculation with Mycorrhizal fungi. In the case of interaction of the level of fertilization 100 with inoculation Mycorrhizal strains, the highest leaf area was recorded in the treatment of a strain.

Entrophos Porainfeqeas: Its value was 33308 cm^2/plant, and the lowest leaf area in the case of a strain ***Scutellospora sp.,*** which is 2508.38 cm^2/plant, reported that the availability of phosphorous in the soil can benefit root architecture, root length, branching, and root hair progress. Thus, the increase in root weight can also be expected due to the rise in carbon mineralization from organic matter decomposition, leading to increased nutrient availability [23].

Table 7. Effect treatment inocula with mycorrhizal strains and with the phosphate fertilization levels on area of leaves/plant cm^2/plant

Mycorrhizae	**Fertilizer P as super phosphate**			**Mycorrhizae average**
	0kg p/ha	50 kg p/ha	100 kg p/ha	
No inocula mycorrhizal	1725.33±217.48	1630.00±78.58	1647.67±17.79	**1667.67±124.00**
Rhizophagus intraradices	2310.00±41.73	1872.67±18.58	3098.00±91.54	**2426.89±540.21**
Claroideoglomus etunicatum	3211.67±145.66	3170.00±113.53	2703.00±61.26	**3028.22±263.22**
Gigspora sp	2757.00±492.95	3583.67±107.08	2806.67±66.58	**3049.11±475.31**
Entrophos Porainfeqeas	2818.00±120.50	2454.33±212.17	3330.00±209.01	**2867.44±413.47**
Scutellospora sp.,	2481.67±108.49	2561.00±130.16	2508.33±87.52	**2517.00±101.56**
Archaeospora trappei	2906.33±71.36	3108.00±59.91	2754.00±168.90	**2922.78±181.52**
funneliformis mosseae	2662.00±108.78	2178.00±93.40	2575.67±106.57	**2471.89±240.75**
Fertilizer P average	**2609.00±463.09**	**2569.71±654.49**	**2677.92±484.19**	
F-value	**For fertilizer P→ 3.033*** **For Mycorrhizae → 80.183***** **For F+M→ 17.191*****			

*** Significant variation at P<0.001

4. Discussion

Previous studies have showed that maize plants inoculated with AMF grow better than non-mycorrhizal plants under salt stress conditions [24]. Results of the present study showed that colonization rates varied among fungal species.

Rhizophagus intraradices collect had the higher rate of root colonization, Mechanisms causing increased growth upon AM fungal inoculation are not fully unclear, but may be due in part to the rhizosphere acidification and enhanced nutrient acquisition [25].

Mycorrhizal species of both ***funneliformis mosseae*** and ***Claroideoglomus etunicatum*** failed to react positively with the plant association to overcome salinity stress.

It is known that different mycorrhizal species have different abilities to influence plant growth under saline conditions. Grow under saline conditions in coastal areas of India indicated that out of 16 different species of mycorrhizas, only ***G. macrocarpum***, ***G. fasciculatum***, and ***Scutellospora corralloides*** helped the trees resist soil salinity and yield better biomass [26].

Fig.1. Effect of mycorrhizal treatment on corn (on the left) with their control (on the right)

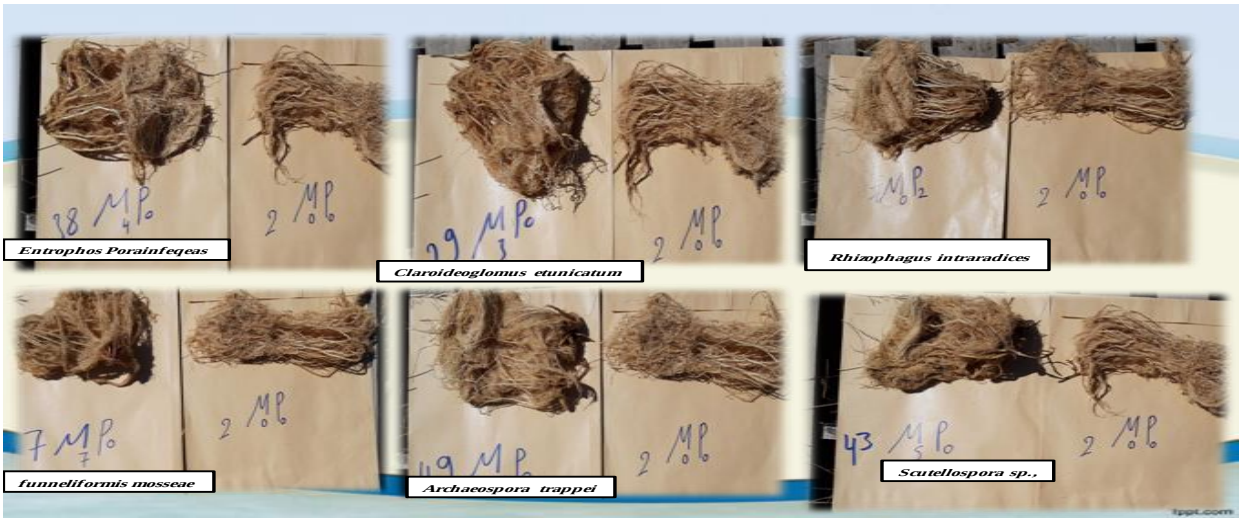

Fig.2. Maize of AMF inoculation on enhanced root structure

Declarations

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Competing Interests Statement

The authors declare no competing financial, professional and personal interests.

Consent for publication

Authors declare that they consented for the publication of this research work.

Availability of data and material

Authors are willing to share the data and material according to the relevant needs.

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