IMPROVEMENT OF LAWN ESTABLISHMENT IN SEMIARID CLIMATE VIA MYCORRHIZAL INOCULUM

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ABSTRACT

There have been many evidences that the mycorrhiza enhance turf grass establishment rate and promote turf stress tolerance levels. However, in Khartoum state, Sudan the public lawns suffer from dryness and hard appearance, as located in semiarid climate. There is very limited scientific research available in turf grass management to support this assertion. This research was designed to study the impact of introducing mycorrhizal inoculum (SYMBIVIT) into soil prior to turf grass establishment. An experiment was conducted at Shamabat in an alkaline soil of the Faculty of Agriculture, University of Khartoum, in order to study the ensuring response. Four treatments consisting of control (uninoculated and without fertilizers, application of P_2O_5 (50 kg ha^{-1}), plus SYMIVIT Mycorrihza inoculum (25Kg ha^{-1}), and mixture of P_2O_5 and SYMBIVT with same doses (50 kg ha^{-1} and 25 kg ha^{-1} respectively).The results showed that the mycorrhiza association in semiarid region improved lawn establishment not only in better turf grass quality via more Freshness with higher water and phosphorus and chlorophyll content but also improved the soil phisco-chemical properties. Furthermore the mycorrhizal inoculum encouraged the growth of other soil micro-organisms and / or enhanced the activity of the indigenous mycorrhiza. Adding Phosphorus with mycorrhiza enhanced these capabilities but it is negatively affected the other microorganisms. More studies are needed to optimize the mycorrhizal inoculum dose under local conditions with other beneficial microorganisms and its reflection on soil composition.

Key words: Mycorrhiza, Mycorrhizal inoculum, turf grass, lawn, semiarid climate

Introduction

The dryness and hard appearance of lawns comes mainly from alkaline soil and the semi arid climate (Habte, 2000). The mycorrihza symbioses have many benefits to plant growth including increased absorption of water and nutrients (N, P, K, Ca, S, Cu, and Zn) (Tinker and Gilden 1983, Ortas, 2010). However, the most prominent and consistent nutritional effect of mycorrhiza fungi is in the improved uptake of immobile nutrients, particularly P, Cu, and Zn (Pacovsky,1986 and Manjunath and Habte, 1988). The fungi enhance immobile nutrient uptake by increasing the absorptive surfaces of the root. Mycorrhizal fungi usually have their maximum effect on host plant growth when the level of P in the soil solution is such that P is barely accessible (Habte, 2000, Smith *et al.*, 2011) which is the case in alkaline soil. The high phosphorus content and uptake in plants is very strong correlated with chlorophyll content and even with habitat (Bouma and Dowling, 1982). In addition, mycorrihza symbioses boosted turf grass acclimation with more dry matter accumulation and freshness and greenish appearance (Petrovic, 1984)

Enhancing soil quality is a prerequest in landscape establishment especially in subtropics and semiarid regions (Habte, 2000). Mycorrihza association play significant role in such dry and alkaline habitats (Raghuwanshi and. Upadhyay, 2010). Mycorrhiza enhances soil physical characteristics (Ianson and Smeenk, 2015) as well as its improvement of chemical composition.

Also, the mycorrhizal association is affected by soil condition and environmental factors (Brundrett, 1991, Van Der Heijden and Scheublin , 2007). The mycorrihza improved soil aggregates, water holding capacity, and the carbon sequestration and as well as enhanced interaction with soil microbiota (Leifheit, 2014), mycorrhiza abundance in the soil and in the root is usually increased (Johnson *et al*., 2003; Parniske, 2008) and probably more easily detected in nutrient poor soils with neutral or alkaline soil pH (Leifheit, 2014).

Ahmed and Abu-elkhair, (2010) found that the turf loans in Shambat area in Sudan with higher mycorrhizal association, had higher freshness and greenness. Furthermore, there was clear evidence that the phosphorus content influenced turf quality and its appearance. Also, they reported a good response to inoculation and superiority in shoot and root fresh and dry matter compared to uninoculated soil. Building on this, therefore, the aim of this study was to investigate the impact of introducing mycorrhizal inoculum (SYMBIVIT) into soil prior to turf grass establishment on turf grass quality and its influence on alkaline soil composition in terms of water, nutrient flow and microbiota in order to achieve sustainable lawn establishment.

Materials and Methods

Two experiments were designed to evaluate the success of SYMVIT mycorrhiza in inoculating turf grass at Shamabat alkaline soil under local conditions. The experiment was carried out at the Faculty of Agriculture, University of Khartoum, Shambat, Sudan (Lat.15°-40° N long 22°-32° E), which is located in semiarid climate characterized by summer rains and warm winter. Average annual rainfall is 121mm falling mainly in July and August and it is erratic in quantity and distribution. the mean annual temperature is 29.9 ° C in a range of 41.5° C in the hottest summer and 16.5° C minimum in winter. The first experiment was a pilot pot experiment conducted for one month with two sets with three pots each, one set was uninoculated and the other one was inoculated with 25Kg ha^{-1}SYMIVIT mycorrihza. SYMIVIT Mycorrihza for turf grass (Symbivit Mycorrhiza produced by: Symbiom, Ltd., Lanskroun 563 01, Czech Republic) .The inoculum dose was adjusted as recommended in labeling package. After one month the existence of mycorrhiza was checked under the microscope after staining .Also, the percentage of infection was calculated according to (Biermann, 1981).

This pilot experiment was followed by a second field experiment to investigate the extent turf grass quality improvement by the introduced mycorrhizal inoculum (SYMIVIT) in the harsh climate and soil conditions and its effect on clay alkaline soil properties of Shambat.

The field experiment was conducted at the Faculty of Agriculture in non cultivated soil. Four treatments, each replicated three times, were adopted in a randomized complete block design. The treatments were uninoculated control, fertilized with P_2O_5 (50kg ha^{-1}), inoculated with SYMIVIT mycorrhiza (25Kg ha^{-1}), and mixture of P_2O_5 fertilizer and SYMBIVT inoculum with the same two doses (50 kg ha^{-1} and 25 kg ha^{-1} respectively). The field was prepared manually by harrowing and leveling, and was divided into plots of 0.4x0.4 m^2. A tap-water hose supplied irrigation water. The soil was irrigated slightly one day before sowing. Turf grass Bermuda (*Cynaden dactylon L.*) seeds were sown (320 kg ha $^{-1}$) according to Lowe's seed calculator (http://www.lowes.com/cd_Seed+Calculator_54331289) in range of California coastal (http://www.californiamycorrhiza.com/landscaping.html) due to the climate similarity. A thin layer of soil was added to cover the seed and then irrigation very slightly four times per days until seeds were germinated then two times per day till the end of experiment (one and half month).

Analytic parameters

Turf grass quality
One and half month later turf grass was harvested, shoot and root matter was estimated.
Leaf phosphorus content was determined by Vanadate-Molybdate-Yellow method (Chapman and Pratt, 1961). The Chlorophyll content was determined colorimetrically on fresh matter base according to Schertz (1928).

Soil properties
Soil analysis was done twice: The first one was initial soil before sawing (1-30 cm depth). The initial soil samples were air dried and ground to pass a 2.00 mm sieve. The second one was done at the end of the experiment (after harvest) for rhizospheric soil of different plot treatments. Since the volume of rhizosphere soil in each replicate was little, a complex samples from the three replicates of each treatment were used for the analyses.

Soil pH was measured by Analogue pH meter WAP. The electrical conductivity of the extract (EC) was obtained by Conductivity Meter WAP CM35. $CaCO_3$ along with EC and pH was measured in the different treatments. The water stable aggregates (WSE) percentage proportion for different treatments was done by methods described by Rillig, *et al*.(2002). Water holding capacity (WHC) and field capacity were determined. The Concentrations of (O) C, N, P, Mg, Ca, K and Na in the soil extracts was also measured. All soil parameters analyses were carried out in the laboratories of the Department of Soil and Environmental sciences, Faculty of Agriculture, University of Khartoum.

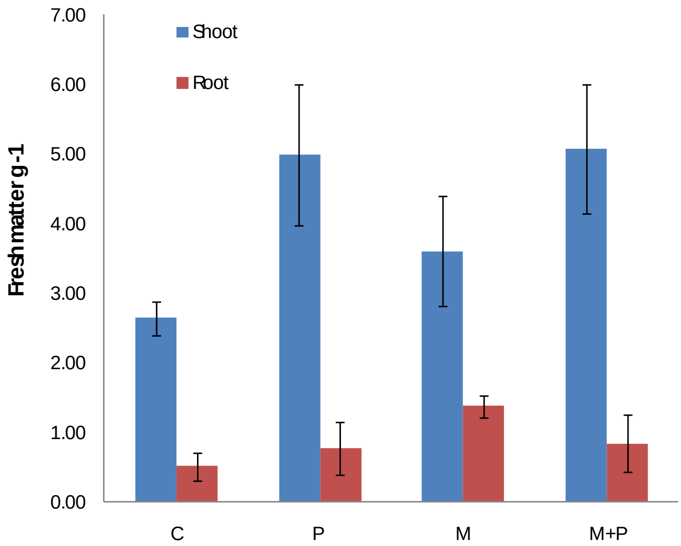
Mycorrhizal infection percentage and total microbial count were made before sowing and after harvesting at the Department of Botany and Agricultural Biotechnology, biofertilizers laboratory. All data calculations and statistical analyses and figures were handled by excel Version2010.

Results and Discussion:

1- The turf grass quality

The response to mycorrihza inoculation was superior in shoot fresh weight compared to the control. However, phosphorus fertilizer enhanced the capabilities of mycorrihza when added together and gave gives the highest shoot fresh weight compared to the treatment when the mycorrhiza inoculum added alone (Fig 1). Similar fndings is reported by Ortas (2010). In contrast, mycorrhizal inoculum was the most superior in root fresh weight which was explained by the extensive mycelium fungal net with root hairs. Nevertheless, shoot fresh weight of treatment inoculated only with mycorrihza did not exceed that of treatment fertilized with chemical P_2O_5, gave the smallest shoot root ratio. This smallest shot root ratio was a good indicator for good establishment for future growth. A high proportion of root production resulted in more water and nutrients uptakes and more climatic adaption to dry semiarid region (Reich, 2002).

Figure 1: The Fresh matter development of one and half month turf grass.. Where C: = control, P = P_2O_5 supply, M = SYMIVIT mycorrhiza M+P = SYMBIVT Mycorrihza +P2O5



Phosphorus content turf grass leaves showed similar trend of shoot fresh weight in response to different treatments (Fig 2) and also the chlorophyll content (Fig 3). The freshness and greenish were indicators for turf grass health (Jeffries *et al*., 2003, Ahmed and Abuelkhair, 2009). The results showed that high phosphorus content and uptake in plants was very strongly correlated with chlorophyll content (Bouma and Dowling, 1982) which was reflected in the greenish appearance of turf grass (Jeffries *et al*., 2003). As phosphorus was very important for chlorophyll stability (Bojović and Stojanović, 2005)

Figure2: Phosphorus content in Shoot of one and half month old turf grass*. Abbreviations as in figure1

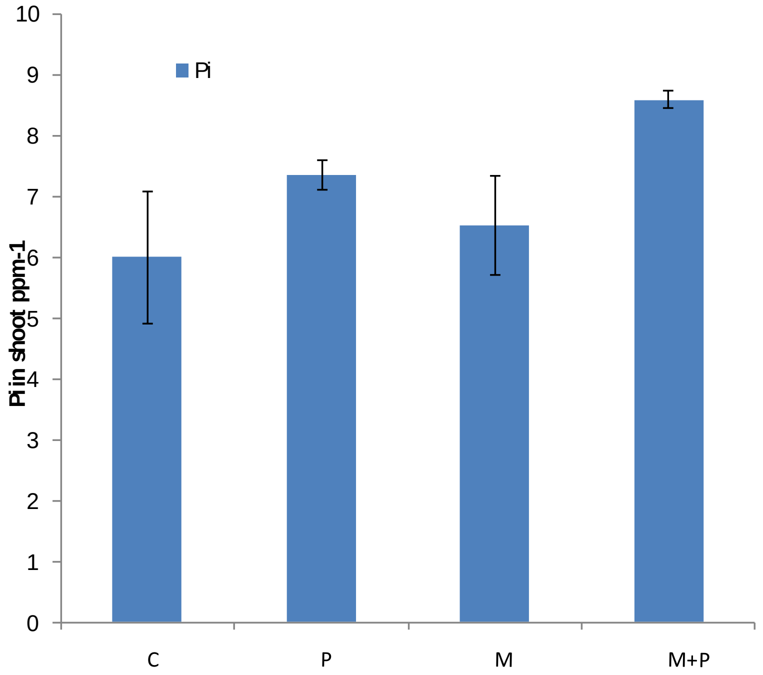
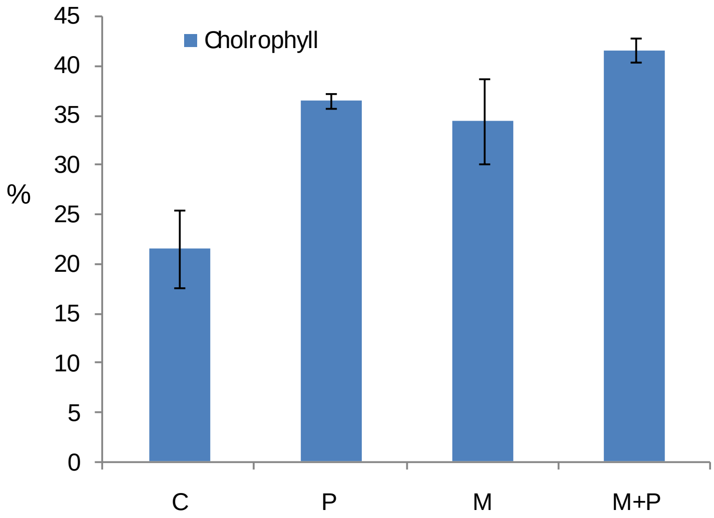


Figure3: The cholophyll content in leaves of one and half month old turf grass*. Abbreviations as in figure 1

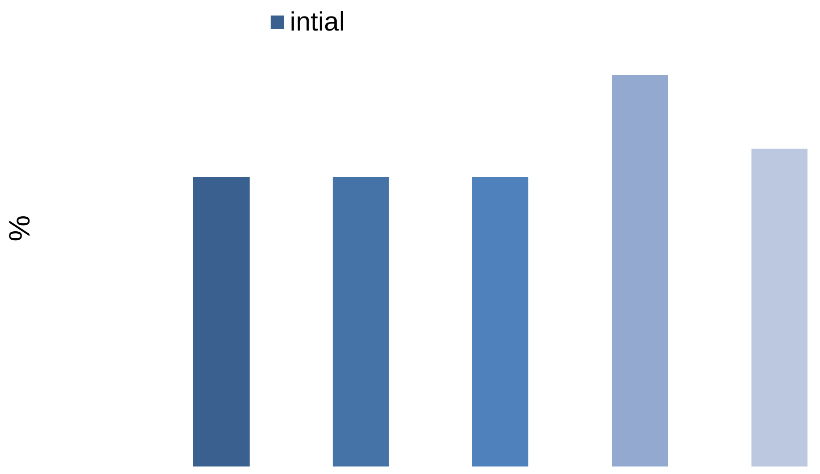


2- The Physical and chemical properties of rhizospheric soil:

2.1- Soil structure aggregates, water holding capacity and buffering capacity and mycorrhizal influence

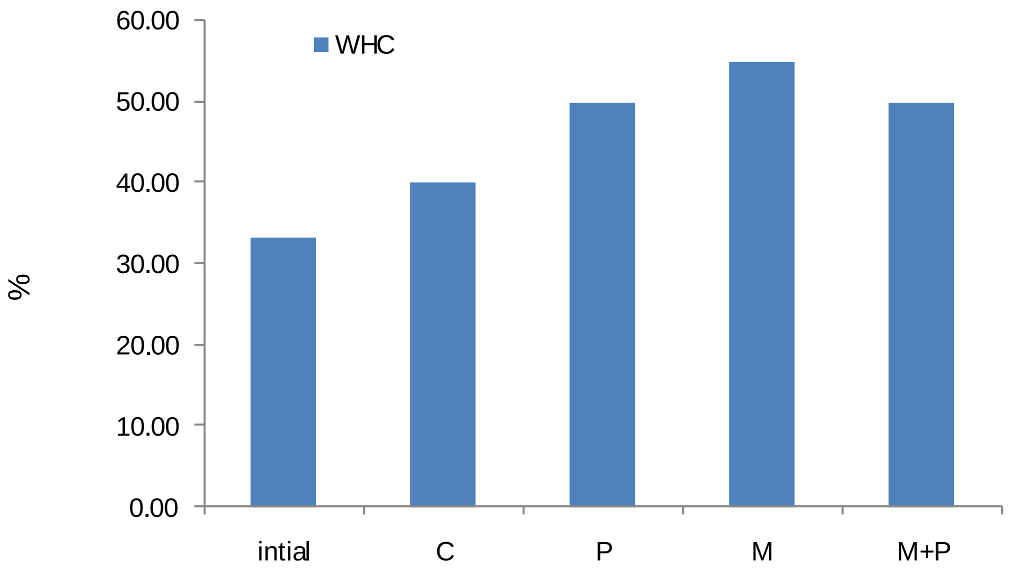
The physical and chemical conditions of rhizosphere soil are known to be considerably differ from those of Bulk soil, because of a range of processes that are induced directly by plant roots and rhizosphere microfolra activities.

Figure4: Effect of different mycorrhizal treatments on the proportion of water stable aggregates (WES) under one and have month old turf grass. Where initial = soil before sawing, C, P, M and M+P as in figure 1.



Mycorrihza influence the soil structure (Miller and Jastrow, 2000) and make more water stable aggregates(Fig.4) due to their excretion of glomolin (Rillig, *et al*., 2002), formation of mycelium (Rillig *et al.*, 2010) and other factors (Leifheit *et al*., 2014). Nevertheless in Fig. 5 this was not very exactly reflected in water holding capacity despite the highest WHC was found in the treatment inoculated with mycorrihza which was attributed to the hyphae fungal net with root hairs as mentioned before (Rillig *et al*., 2010) and this can also be correlated strongly with root fresh weight .

Figure5: Effect of different mycorrhizal treatments on water holding capcity of soil (WHC under one and have month old turf grass. Where initial = Soil before sawing, C, P, M and M+P as in figure 1.



2.2-Soil chemical properties with special allusion to phosphorus

Table1: Effect of different mycorrhizal treatments on the rhizosphere soil H^+ buffering capacity as related to the total soil $CaCO_3$ content and pH Change induced by one and half month old turf grass. Where initial = Soil before sawing, C, P, M and M+P as in figure 1.

	$CaCO_3$ %	EC ds m^{-1}	Initial	pH Final	H^+ M mol g^{-1} dm
Initial	4.43	1.3	7.66	_	_

C	4.7	0.5	7.66	7.45	1.62
P	4.78	0.5	7.66	7.45	1.62
M	5.22	0.4	7.66	7.24	2.63
M+P	4.61	0.6	7.66	7.61	1.12

There was a slight acidification as a result of proton flux (H^+) in the rhizosphere soil accompanying the plant growth. The H^+ flux was the highest with mycorrhizal inoculation alone (table1). Nevertheless, when added together with P_2O_5 fertilizer it resulted in the lowest acidification. In addition to proton fluxes from root and microorganisms exudates, release of CO_2 by respiring roots, mycorrihza and other soil microorganisms was likely to cause stronger acidification of the rhizosphere near root apices where respiration was most rapid. The slight acidification was accompanied with the increase of $CaCO_3$ concentrations which was reflected in the greater decrease of EC (table1). However this acidification was reported for alkaline soil (Hinsinger, 2001). This acidification comes from root, mycorrihza and other microorganisms' exudates mainly organic acids (Rillig, *et al*., 2002) and glomolins (Rillig, *et al*., 2002). This may explain how the mycorrihza exudates help in salinity and alkalinity tolerance

Table2: Chemical composition of rhizospheric soil of the different treatments induced by one and half month old turf grass. Where initial = Soil before sawing, C, P, M and M+P as in figure 1.

	(O) C %	N %	P %	Ca Meq L^{-1}	Mg Meq L^{-1}	Na Meq L^{-1}	K Meq L^{-1}
Initial	0.88	0.07	0.99	10.80	5.20	10.00	38.40
C	0.24	0.03	0.04	5.00	2.00	0.37	0.18
P	0.40	0.03	0.05	5.00	2.00	0.38	0.20
M	1.52	0.14	0.05	3.00	4.00	0.31	0.25
M+P	0.80	0.07	0.05	4.00	4.00	0.37	0.20

The depletion of soil organic carbon and nitrogen content in the rhizosphere was known (Hinsinger, 2001). The highest organic carbon and nitrogen were found with mycorrhizal inoculation alone (Table2). The increment of organic carbon and nitrogen content strongly correlated with water stable soil aggregates (R^2=0.78 and 0.86 respectively) (Leifheit *et al*.2014). Furthermore the organic carbon and nitrogen response was similar to H^+ flux .These may have been due to exudates from roots, mycorrhiza and microorganisms (Borie *et al*., 2008).

A steep decrease in the concentration of P anions in the rhizosphere was found compared to the initial soil sampling (Table2), generating concentration gradients that force P diffusion towards the roots (Hinsinger, 2001). However still P concentration in rhizosphere given phosphorus fertilizer supply (without or with Mycorrihza), showed higher concentration compared to the control. The mycorrhizal inoculum alone resulted in the same P concentration as addition of phosphorus fertilizer. Habte, (2000) reported that mycorrihza in subtropical soil resulted in a phosphorus concentration in soil solution similar to phosphorus fertilizer .In addition, the concentration of Pi in rhizospheric soil solution can increase due to the reduction of the pH by mycorrhizal inoculation (table 1 and 2)

P ions are important inorganic anions in the soil solution and thus have a strong tendency to form ion pairs with several metal cations, most notably with Ca and Mg in the alkaline soil (Lindsay, 1979).In initial soil sampling Ca and lesser Mg, were dominants in soil solution. The mycorrhizal inoculation usually reduces Ca concentration in the rhizosphere which is it not in case of Mg (Table2) .Ca is known to bind P, therefore the reduction in Ca concentration enhanced the release of Pi in soil solution observed in this study. Furthermore mycorrhizal inoculation changes the Ca: Mg ratio .On the other hand, the same trend of Ca: Mg ration applies for Na: K ratio. Bivalent cations such as Ca and Mg also improve soil aggregation by promoting soil organic C incorporation conferring aggregate stability (Borie *et al*., 2008). Also, this can be correlated strongly with EC and pH alteration which indicated that phosphorus extracted from clay minerals in soil solution by more acidification with mycorrhizal exudates over and above root exudates (Hinsinger, 2001).

2.3- Soil Microbiota:

In the pilot pot experiment, Bermuda turf grass grown for one month showed 60% infection when treated with SYMVIT Mycorrihza in compared to non treated Bermuda turf grass which showed only 10% infection

Turf grass in this alkaline soil showed strong mycorrhizal infection in all treatments even the control and with adding only chemical P_2O_5 fertilizer (with indigenous mycorrihzae 25% infection) (table3) and over 60 % when is inoculated with SYMVIT mycorrihza with or without adding chemical P_2O_5. Similar results obtained by Raghuwanshi and. Upadhyay, (2010) sowing that plants inhabiting alkaline showed strong mycorrhizal dependency. Despite the existence of indigenous mycorrihza, turf grass showed very good response to the introduced inoculum SYMVIT which may reflect the need for further inoculation with mycorrihza perhaps of higher dose than those applied in this investigation.

Table3: Quantification of bacteria (CFU) and mycorrhizal infection (%) of the different treatments induced by one and half month old turf grass. Where initial = Soil before sawing, C, P, M and M+P as in figure 1.

	Bacterial plate count CFU g^{-1}	Mycorrhizal root infection %
Initial	<30	*detected
C	82 X10^{3}	25
P	80 X10^{3}	25
M	> 300	60
M+P	70X10^{3}	60

The pilot experiment and an earlier study by Ahmed and Abuelkhair (2009), point to results similar to present findings. Soil in this study even in undisturbed soils could have inherently low levels of mycorrhizal fungi and / or its indigenous mycorrhizal fungi may have low efficiency, and thus they will need to be preempted by more aggressive, highly effective mycorrhizal inocula (Habte and Fox 1989). The mycorrihza role is more extended in turf grass establishment in Sudanese alkaline soil agricultural systems as Habte, (2000) reported that alkaline soil in subtropical regions have high soil P-fixing capacities and therefore, Mycorrihza exploit this P and make it available in soil solution. Poor farmers in such regions like Sudan are, therefore, spread the high cost of P fertilizers. In addition mycorrihza association enhanced the tolerance of plants under stressful condition of semiarid climate (Raghuwanshi and. Upadhyay, 2010).

Adding mycorrhiza inoculum not only encourages the mycorrhizal symbiosis but also the rhizosphere bacteria (RB) which has the most abundant bacterial number as shown in table 3. Similar results were reported by Filion *et al*., (1999). P_2O_5 fertilizer discourages the growth of the RB while it encourages the mycorrhizal symbioses. This highlights for making some available Pi to establish this symbioses (Borie *et al*., 2008, Ortas, 2010). This high abundance of RB with mycorrihza inoculation is another confirmation for the richness of H^+ flux, organic carbon and nitrogen percentage in rhizosphere soil and also better soil aggregates (Tisdall, 1994).This finding also confirms the interaction of mycorrhiza with other microorganisms in soil aggregation and could indicate an important role for mycorrhiza in soil C storage (Leifheit, 2014)

Conclusion

The endo -roots mycorrhiza associated with turf grass even in the dry conditions of the Sudan improved the grass growth and its appearance through good shoot: root ratio, more freshness and greenish. In addition the mycorrhiza association improved the water and nutrients uptake in alkaline soil through the improvement of soil aggregates and chemical composition specially P, C storage, N content and better Ca: Mg and Na: K ratio with its exudates and their mycelium spread net. Mycorrihzae symbioses encouraged the growth of other soil micro-organisms and/ or enhanced the activity of the indigenous mycorrhiza which improved sustainable turf grass establishment. Further investigation is curical to optimize the mycorrhizal inoculum dose under local conditions with other beneficial microorganisms and its reflection on soil composition toward better sustainable turf grass establishment.

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