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Efficiency Of Mycorrhiza-Forming Fungi Associated With Panicum Maximum Cv. Mombasa

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ABSTRACT

The objective of the present study was to characterize the diversity of morphospecies of arbuscular mycorrhizal-forming fungi (AMF) associated with rhizosphere of Mombasa (Panicum maximum cv. Mombasa) pasture cultivation in Sampues María, Sucre, Colombia and bioaugmentation of AMF spores in trap crops with rice seedlings. The sampling was carried out in the rhizosphere of Mombasa plantations in the municipality Sampues, department of Sucre, Colombia. Spores, separation and identification of AMF morphospecies were isolated; biaumentation of spores was performed in trap culture and the growth capacity of AMF was evaluated. A total of 67 morphospecies were distributed in 9 genera: Glomus, Acaulospora, Ambispora, Sclerocystis, Diversispora, Funneliformis, Scutellospora and Entrophospora. Treatments with Glomus geosporum, Glomus multicaule, Glomus agreatum and the AMF consortium induce chlorophyll production, increased stem length and bioaugmentation of spores / g of soil. The individual and consortium AMF morphospecies become an alternative for the production of bio-inputs with these fungi for their use and the promotion of growth in of Mombasa pasture on livestock farms in the department of Sucre.

Keywords: fungi, rhizosphere, plants, nutrition, growth**.**

INTRODUCTION

Seasonal and annual growth and yield differences in morphological and physiological components in grass are a direct function of climatic conditions, soil fertility and biological indicators, and management practices (Ramírez et al., 2011). Plant biomass utilisation depends on the proportion of leaves, stems and roots that are generated by genotype-

environment interaction; these components result in forage yield (Njaru et al., 2014, Velasco et al., 2018).

A cattle farming in the department of Sucre, Colombia, occupies 768600 ha of pasture representing 13.7% of the livestock area of the Caribbean region (Pérez et al., 2018). In addition, dual-purpose mode represents the main economic activity in the department of Sucre, where 94.9% of the total area destined to livestock activity is exclusively dedicated to cattle grazing (Pérez et al., 2018). Pasture and forage production in Colombia is mainly for livestock use, as a source of feed for livestock (Pérez et al., 2018).

Mombasa grass production in the Colombian Caribbean region is widely distributed, covering extensive areas of the tropical dry forest (bs-T) and very dry tropical forest (bms-T) life zones in the departments of Córdoba, Sucre, Bolívar and Magdalena; However, due to physiographic factors, anthropogenic actions degenerative of the environment and the use of inadequate technologies, have brought as a consequence, the degradation of the physical, chemical and biological properties of the soil, which has limited the supply and quality of this pasture mainly in dry season (Pérez et al. , 2010).

In tropical regions there are low rates of animal production, caused by the absence of sustainable nutrition practices, seasonal distribution of rainfall and high temperatures. These factors affect productivity and induce changes in the chemical composition of dry matter (DM) in plant components and yield, such as at the end of the rainy season when more reproductive stem development is observed. In addition, there is an increase in the abscission of structures, especially leaves, and the presence of undesirable plants (Ramírez et al., 2009).

In the aforementioned situation and without the use of adequate management practices in pasture soils, 84% of the cattle in Southeast Mexico are found (Ramírez et al., 2009) and in the search for alternatives to improve the quantity and quality of the biomass in this ecosystem, P. máximum Jacq. cv Mombasa released in 1993 by the Centro Nacional de Pesquisa de Gado de Corte (CNPG), Brazil, was introduced in the region. However, despite its nutritional quality, and remarkable production potential (22.45-33 t ha-1) reported in Central and South America (Da Silva et al., 2010).

Faced with this situation, the use of chemical fertilisers becomes an alternative to overcome this difficulty, which improves pasture productivity, but causes an imbalance in the populations of native soil microorganisms, which fulfil important functions within ecosystems such as: nutrient supply, moisture retention, improved soil structure, among others (Lara et al., 2011). In this sense, as an alternative to mitigate the effect of fertilisers in recent years, several studies conducted with arbuscular mycorrhiza-forming fungi associated with colosoana grass species (Pérez et al., 2003; 2010; 2012; 2016) and angelica grass (Pérez et al., 2013; 2015; 2016), show an important potential for plant nutrition.

Arbuscular Mycorrhizal fungi (AMF), belonging to the phylum Glomeromycota (Schüβler, et al., 2001), are soil microorganisms that form symbiotic associations with approximately 80% of terrestrial plants found in almost all terrestrial ecosystems (Smith & Read, 2008; Carreón-Abud et al., 2013), forming arbusculi where carbon and phosphorous exchange takes place between the fungus and the plant, vesicles and hyphae within the cortical cells of the colonizing plants (Strullu -Derrien, et al, 2007), which fulfill the function of storing reserves for the fungus (Cuenca, et al., 2007), this symbiosis generates a strong influence on the growth and productivity of plants (Van der Heijden et al., 2015).

Given the importance of arbuscular mycorrhizal fungi in the fertility, sustainability and growth promotion of pastures, the objective was to evaluate the efficiency of arbuscular mycorrhizae associated with rhizosphere of Mombasa grass from cattle farms in the municipality of Sampués in the department of Sucre, as a biological alternative for in situ use and agrosustainable management of this pasture as a source of animal feed in the Colombian Caribbean and food security of the animal protein produced.

MATERIALS AND METHODS

Study site. The study was carried out in livestock farms located in the municipality of Sampues sown only with Mombasa grass, on which an inventory of the diversity of arbuscular mycorrhizal fungi was carried out for their possible efficiency in the production and sustainability of this pasture.

Collection and processing of samples. A random zig-zag sampling was carried out in each sidewalk, taking between 15 to 20 random subsamples at a depth of 0-20 cm, collecting at the same time with a hole and soil and roots. Subsamples were obtained from each sampling, which were homogenized to make up one sample per farm with an approximate weight of 2000 gr., They were deposited in plastic bags labeled with the name of the village and the respective coordinates. The samples labeled in plastic bags were taken to the laboratory and stored at 4 ° C until further analysis. For the isolation of spores, the samples collected in the field were sieved with a set of sieves made up of 180, 150 and 38 µm Mayas, respectively, to separate the thick parts of the soil (stones, gravel) and roots (Pérez et al., 2012; Pérez and Peroza, 2013).

Isolation and identification of spores. The AMF spores were isolated following the protocol proposed by (Pérez et al., 2012; Pérez and Peroza, 2013), which consists of creating a density gradient using a 50% sucrose solution, later the samples are centrifuged at 3000 rpm for 5 minutes. The spores were recovered with the 38 µm spore sieve and deposited in Petri dishes, then they were observed under the stereoscope and with the help of a dissecting needle, morphotypes were grouped, taking into account the similarity, color and size of the spores. With the help of a micropipette, the spores of the different morphotypes found were extracted, placed in eppendorf tubes with sterile water, labeled with the number of the farm

where they were isolated and kept in a refrigerator at 4 ° C for 3 days for subsequent identification.

Spore count. It was carried out by using a nematode counting camera, the average number of spores / gr of soil was counted, for which a volume of 2 ml was added to the camera, observations were made under the microscope at 40 x, performing three counts per sample to obtain an average value (Pérez et al., 2012; Pérez and Peroza, 2013).

Morphospecies identification. The isolated morphotypes were deposited in Petri dishes, they were observed under the stereoscope to detail their characteristics in water, verify and eliminate spores of other morphotypes and contaminating particles. Once the spores were cleaned and the morphotypes were verified, they were identified at the genus level, using internationally recognized techniques established by the International collection of Arbuscular & vesicular Arbuscular Mycorrhizal Fungi (INVAM) (International culture collection of vesicular arbuscular mycorrhizal fungi. 2017).

Trap plant inoculation. The AMF spores were morphologically characterized and the 5 morphospecies with the highest abundances were selected for inoculation in the trap plant. For the establishment of the trap plant, the proposed protocols (Pérez, 2003) were used, which were modified and consisted of taking pre-germinated rice varieties of the Fedearroz 2000 variety in wet napkins, which were sown in polyethylene containers (styrofoam) filled with 200 gr of previously autoclaved soil. The treatments used were: 1 (Glomus geosporum), 2 (Glomus multicaule), 3 (Glomus agreatum), 4 (Funneliformis geosporum), 5 (Acaulospora denticulate), 6 (consortium, mixture of treatments 1 to 5) and (control - without HMA spores). The experimental design was composed of 7 treatments with 5 repetitions per treatment. When the seeds germinated (approximately 5 days), the inoculation was carried out with approximately 100 spores of AMF using a 100 µL pipetman brand micropipette, for treatments 1 to 5. For the consortium treatment, 20 spores of the treatments 1 to 5 for a total of 100 spores. The control treatment was added only sterile water.

The parameters evaluated were stem length and chlorophyll measurement. The length was measured in cm, every 10 days after inoculation of the AMF spores. Chlorophyll measurement was performed with the SPAD 502 & 502DL portable chlorophyll meter every 10 days after germination, every 10 days after inoculation of AMF spores. 45 days after the establishment of the crop, the plants were subjected to water stress for 10 days, then they were pruned and the spore / g of soil was counted. To obtain the bioaugmentation data, 50 g of soil were processed in each treatment for isolation and spore counting following the methodology proposed by (Pérez, 2003).

Statistical test. The data obtained in the HMA inoculation setup in plant in trap plants were analyzed with a double ANOVA to determine if there is an influence of the evaluated factors (morphospecies inoculated) on the variables considered. Likewise, a variance component test allowed quantifying the effect on the response variables of each of the factors. In addition, it

was determined if there were significant differences between each of the treatments. For this, comparison tests were carried out using the Kruskal-Wallis method. Finally, a multiple range test was run, in order to group the treatments according to the value of the observed means. All tests performed in this trial were run using the software (Statgraphics Centurion XVIII (Statpoint Technologies Inc. 2017).

RESULTS

A total of 67 morphospecies were isolated, of which 47 were found in the municipality Sampues (Table 1) (Figure 1). The morphospecies were taxonomically classified into 9 genera: Glomus, Acaulospora, Ambispora, Sclerocystis, Diversispora, Funneliformis, Scutellospora and Entrophospora. Of which 62% correspond to the Glomus genus, followed by the Acaulospora genus with 15%, Ambispora 7%, Sclerocystis with 5%, and a percentage of 3% corresponding to the genera Diversispora, Funneliformis and Scutellospora. For the genera Entrophospora and Asparagus, a percentage of 1% was found (Figure 2).

Table 1. Morphospecies of AMF isolated from the rhizosphere of Mombasa pasture in the municipalities of Sampues, departament of Sucre, Colombia.

N°	Morphospecies	N°	Morphospecies
1	Acaulospora denticulate Sieverd. & S.Toro (1987)	35	Funneliformis sp
2	Acaulospora excavata Ingleby & C.Walker (1994)	36	Funneliformis geosporum (T.H. Nicolson & Gerd.) C. Walker & A.Schüßler
3	Acaulospora laevis Gerd. & Trappe	37	Glomus caledonium (T.H. Nicolson & Gerd.) Trappe & Gerd
4	Acaulospora sp.	38	Glomus viscosum T.H. Nicolson
5	Acaulospora sp.	39	Glomus lamellosum Dalpé, Koske & Tews
6	Acaulospora sp.	40	Glomus macrocarpum Tul. & C. Tul. 1845
7	Acaulospora sp.	41	Glomus sp.
8	Acaulospora sp.	42	Glomus sp.
9	Acaulospora sp.	43	Glomus claroideum N.C. Schenck & G.S. Sm
10	Acaulospora sp.	44	Glomus hoi S.M. Berch &Trappe
11	Ambispora appendicular C. Walker	45	Glomus intraradices C. Walker & A. Schuessler 2010
12	Ambispora gerdemannii C. Walker, Vestberg & Schuessler	46	Glomus luteum L.J. Kenn., J.C. Stutz & J.B. Morton
13	Ambispora sp.	47	Glomus manihotis R.H. Howeler, Sieverd. & N.C. Schenck 1984
14	Asparagus sp.	48	Glomus occultum C. Walker 1982
15	Ambispora sp.	49	Scutellospora sp.

16	Diversispora spurca, C. Walker & A. Schüßler 2004	50	Sclerocystis sp.
17	Entrophospora nevadensis Palenz., N. Ferrol, Azcón-Aguilar & Oehl, 2010	51	Sclerocystis sp.
18	Glomus multicaule Gerd. & Bakshib	52	Glomus sp.
19	Glomus aggregatum Schenck & Smith, 1985	53	Glomus sp.
20	Glomus ambisporum, G.S. Sm. & N.C. Schenck, 1985.	54	Glomus sp.
21	Glomus cerebriforme, McGee, Trans. Br, 1986.	55	Glomus sp.
22	Glomus citrícola D.Z. Tang & M. Zang 1984	56	Glomus sp.
23	Glomus clarum Nicolson & Schenck, 1979	57	Glomus sp.
24	Glomus etunicatum Becker & Gerdermann, 1977	58	Glomus sp.
25	Glomus fasciculatum Gerd emann & Trappe, 1974	59	Glomus sp.
26	Glomus fecundisporum Schenck & Smith, 1982	60	Glomus sp.
27	Glomus geosporum(T.H. Nicolson & Gerd.) C. Walker 1982	61	Glomus sp.
28	Glomus occultum Walker 1982	62	Glomus sp.
29	Glomus radiatum Trappe & Gerdemann, 1974	63	Glomus sp.
30	Glomus tortuosum N.C. Schenck & G.Sm	64	Glomus sp.
31	Sclerocystis rubiformis Gerd & Trappee	65	Glomus sp.
32	Scutellospora calospora (Nicol. & Gerd.) Walker & Sanders 1994	66	Glomus sp.
33	Diversispora aurantium C. Walker & A. Schüßle	67	Glomus sp.
34	Funneliformis sp		

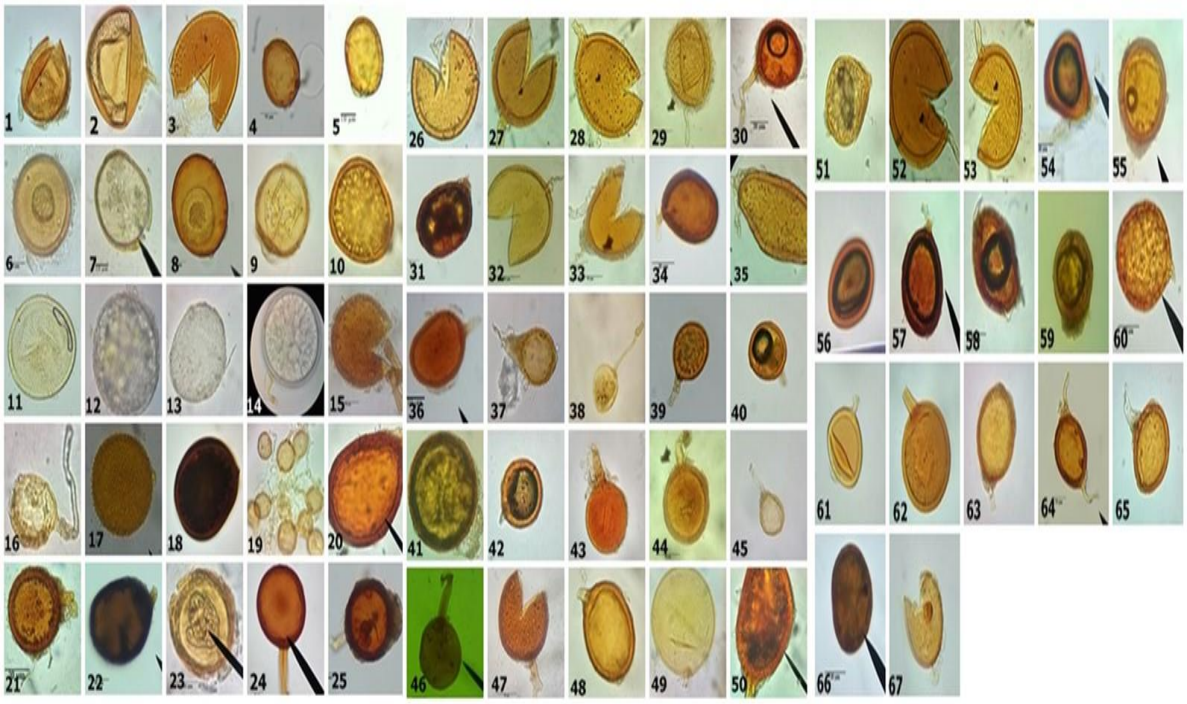

Figure 1. Diversity of AMF morphospecies isolated from rhosphere of Mombasa grass. 1-67: morphospecies of AMF isolated and identified according to results presented in Table 1. 40X, Photo: Chamorro and Pérez, 2017.

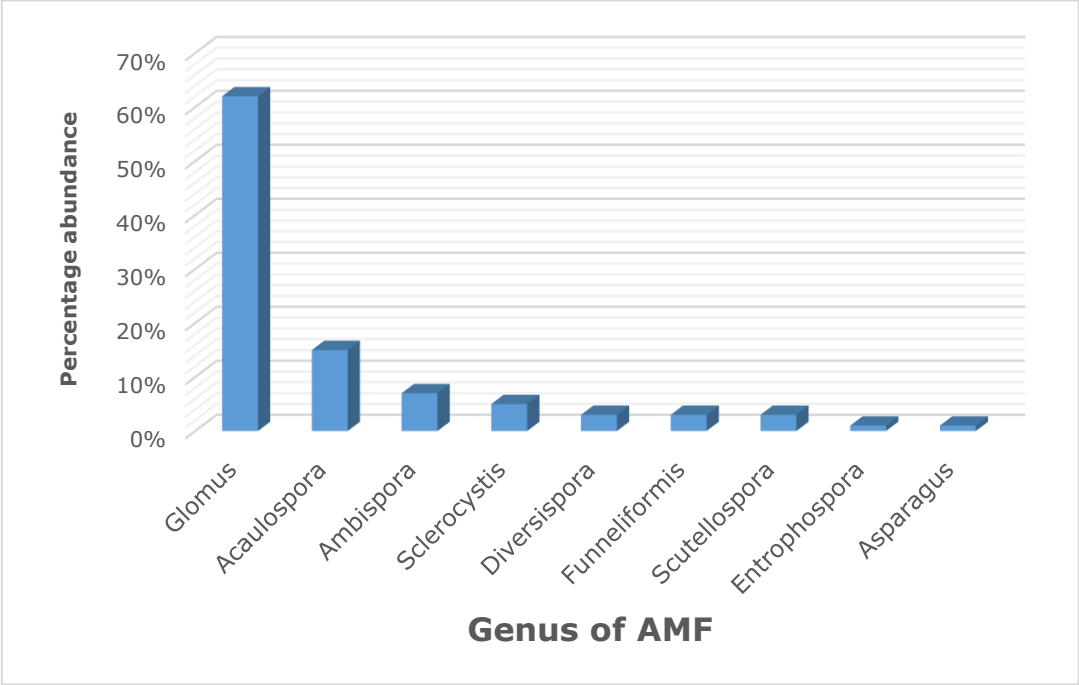

Figure 2. Percentage distribution of morphospecies by isolated genera of AMF in the rhizosphere of the Mombasa pasture in municipalities of Sampues, Sucre, Colombia.

Regarding the amount of spores / g of soil, associated with the rhizosphere of Mombasa pasture plantations in the municipality of Sampues, it presented the maximum amount of spores 624 / g of soil, with respect to sheep where values of 483 spores / g were reported. In the trap inoculation test in rice plants, seven treatments were used as follows: 1 (Glomus geosporum), 2 (Glomus multicaule), 3 (Glomus agreatum), 4 (Funneliformis geosporum), 5 (Acaulospora denticulate), 6 (consortium, mixture of treatments 1 to 5) and (control - no AMF spores). Regarding the variable stem length (Figure 3A) and chlorophyll production (Figure 3B), it was found that the maximum means of both parameters measured at 15, 25, 35 and 45 days after the start of the experiment, showed that the treatments 1 (Glomus geosporum), treatment 2 (Glomus multicaule) and consosrcio, presented the highest values in both parameters, with respect to treatments 3 (Glomus aggreatum), 4 (Funneliformis geosporum) and 5 (Acaulospora denticulate) who presented intermediate values. ; and the lowest values were found in the control treatment (Without AMF), during the experiment (Figure 3A and B).

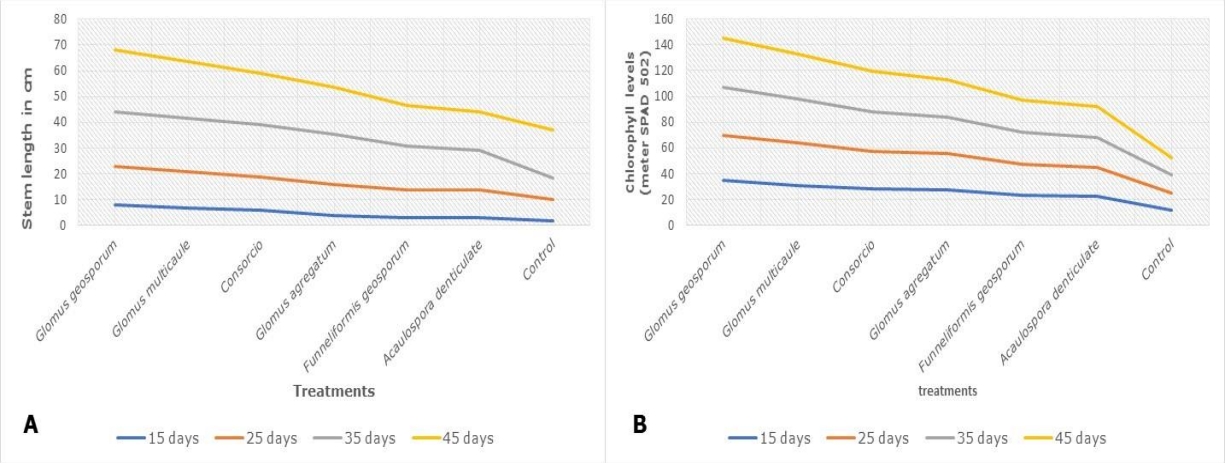

Figure 3. Results of productive parameters of AMF inoculated in a rice plant of the Fedearroz 2000 variety. A. Stem length in cm and B. Chlorophyll levels.

The inoculation tests in rice plants (number of spores / g of soil), shows that Glomus geosporum had the highest rate of spore bioaugmentation going from 100 spores initially inoculated to obtain 456 spores / g of soil at the end of the experiment , in the remaining treatments with (Glomus multicaule, Consortium, Glomus agreatum) there were a number of spores of 389, 368, 323 spores / g of soil, respectively. While for the morphospecies of Funneliformis geosporum and Acaulospora denticulate, the lowest number of spores / g of soil was presented at the end of the experiment with 230 and 225, respectively (Figure 4).

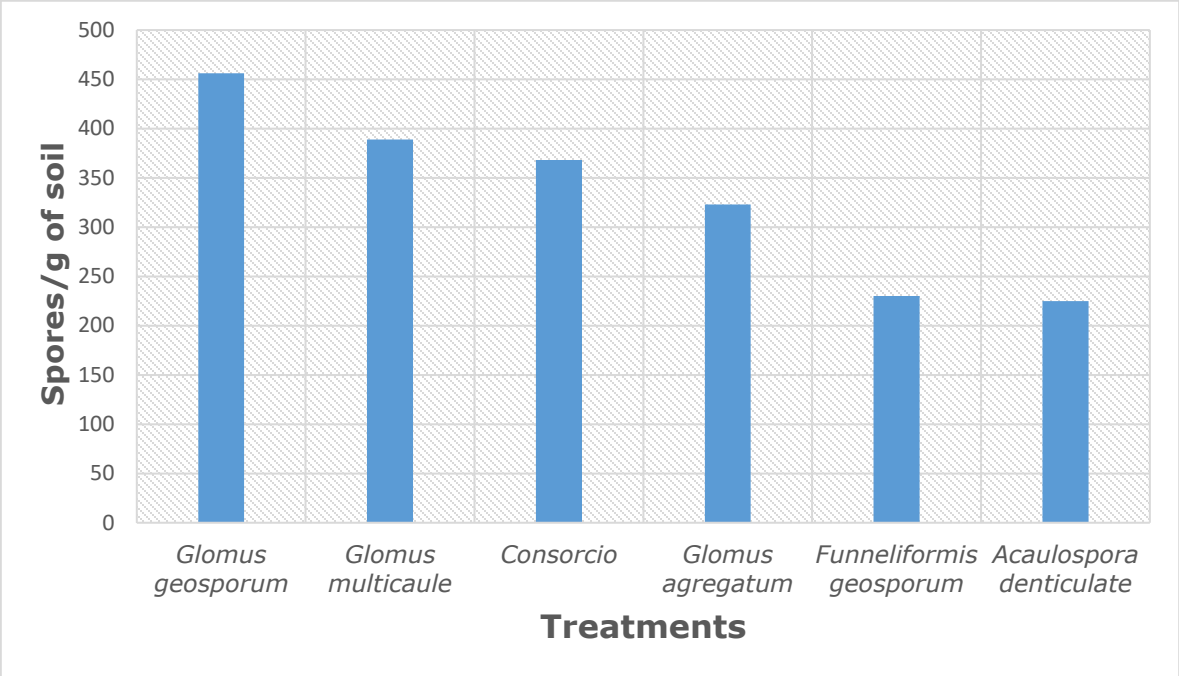

Figure 4. Results of amount of spores / g of soil of bio-increased AMF morphoepecies in trap plant trials with the rice variety Fedearroz 2000.

DISCUSSION

Regarding the diversity of isolated AMF in Mombasa pasture in the municipalities of Sampues, department of Sucre, 67 morphospecies were found located in the genera: Glomus, Acaulospora, Ambispora, Sclerocystis, Diversispora, Funneliformis, Scutellospora and Entrophospora. Study carried out by (Bárcenas et al., 2006) reports the predominance in number of the genera of arbuscular mycorrhizae Glomus sp, Acaulospora sp and Scutellospora sp, the first two coincide with the few works that have been reported on mycorrhizae in Mombasa pasture worldwide. In the study carried out in California and Israel, they are the only ones recorded where the Glomus genus stands out for its abundance (Hass and Menge, 1990).

The results obtained in the bioaugmentation test, in this work, is a point of reference, for the use of HMA, as an alternative as a source of biological fertilizers. Although it is known that plant growth and photosynthesis depend on various environmental factors and on the availability of nutrients, the presence of AMF associated with plants plays an important role in these processes (Vargas, et al., 2010).

CONCLUSION.

The Fedearroz 2000 variety rice trap plant test for AMF isolated from roots of Mombasa pasture indicate that AMF can provide benefits to this plant species, evidencing a greater growth and improvement in the photosynthetic rate, which could increase production if It

will be fertilized with native arbuscular mycorrhizae, since the efficiency of the roots in the Mombasa is very limited by the lack of absorbent hairs, the use of AMF constitutes an alternative to improve this condition, in relation to the a Mombasa grass, and the evidence that has indicate that there are positive responses to the use of arbuscular mycorrhizal fungi, which in addition to increasing the absorption of nutrients (phosphorus, zinc and copper) substantially improve water relations, which translates into a higher growth rate of the plant . The Glomus genus turned out to be the most abundant and with the greatest distribution, adapted to the edaphoclimatic conditions of the study area. Rice seedlings are an option as a trap plant for bio-increasing AMF spores from the soil of Mombasa grass and it is an alternative for the production of inocula of these fungi for use as biofertilizers in these crops in the department of Sucre.

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