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Efficiencies of arbuscular mycorrhizal fungi for agro-sustainable production on livestock farms

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Abstract
The objective was to evaluate in the field the efficiency of native arbuscular mycorrhizal fungi associated with forage production in the grass species Bothriochloa pertusa (L) A. Camus in livestock farms in the municipality of Corozal, department of Sucre, Colombia. The work consisted of three stages: laboratory, greenhouse and on-farm efficiency test. During the on-farm efficiency test, the production of green forage, dry matter, nutritional content of the grass species, spore density and infection percentage were evaluated. The number of spores/100 g soil and the percentage of root infection found on the cattle farms ranged from 2600 and 41 - 65 respectively. 23 morphospecies were isolated of arbuscular mycorrhizal fungi were isolated, distributed in two genera Glomus and Gigospora, with species of the genus Glomus predominating. The 8 morphotypes evaluated in the greenhouse showed different degrees of colonization on Brachiaria decumbens roots. Morphospecies 5, 18 and 22 showed the highest spore densities/ 100 grams of soil. The results show that the morphotypes tested in sterile soils showed high efficiency in forage production in colosoana grass, high phosphorus concentration in plant tissues, high spore density and percentage of colonization in roots, results very similar to the effects of the mixture of morphotypes in natural soils.

Keywords: Mycorrhizae, soil, pasture, growth promotion.

1. INTRODUCTION

The kikuyina grass (Bothriochloa pertusa (L). A. Camus), represents the largest sown area in the department of Sucre, reaching an extension of 274,005 hectares (Ha), distributed in 19 municipalities. Corozal has the largest area planted with this species in the region, representing 32,223 hectares. The soils present different degrees of compaction, erosion problems, low fertility levels, no fertilization, extensive grazing and degraded grasslands. Seasonal rainfall results in a shortage or total lack of fodder during the dry season.

Sustainability of both natural ecosystems and agro-ecosystems is nowadays considered to depend mainly on the balance between the biological components of the soil. In fact, it is accepted that the general aim of the current trend in soil microbiology research is the study of micro-organisms from the ecological, genetic, biochemical and physiological point of view in relation to plant nutrition and protection (Barea, 2002).

There are reports indicating that arbuscular mycorrhizae play an important role in improving soil physico-chemical conditions; they stimulate the growth and nutritional quality of plant species, making them more tolerant to adverse abiotic and biotic conditions. It has been shown that arbuscular mycorrhizae improve production, nutritional quality and increase the tolerance of grass species to trampling, due to the supply of nutrients to the plant, which stimulate continuous regrowth and rapid recovery after defoliation by herbivorous animals (Salamanca, 1999).

Among the most visible benefits of arbuscular mycorrhiza formation is the ability of the fungi to stimulate host plants to increase seed size and production through the incorporation of phosphorus and other nutrients (Montaño et al., 2009). In addition, it is known that the production of phytohormone by the fungus improves soil structure and favors resistance to pests and drought (Jiménez, 2009). In the soil, the mycelium of arbuscular mycorrhizal fungi participates in the formation of aggregates through the adhesion of glomalin particles, contributing to give it structure and stability, reducing erosion and improving the soil's water retention capacity (Ruiz et al., 2011). For this reason, the effects at the soil level are key to the maintenance of plant diversity and soil microorganisms, productivity and the restoration of disturbed ecosystems (Montaño et al., 2009).

Based on the importance of arbuscular mycorrhizal fungi for the agrosustainability of crops, it was proposed as a strategy to isolate and evaluate in the field the efficiency of arbuscular mycorrhizal fungi on the productivity of colosoana grass in field conditions in the municipality of Corozal, department of Sucre, Colombia.

2. MATERIALS AND METHODS

Sampling site and study area

The present work was carried out from the first semester of 2002, in cattle farms, located in the municipality of Corozal, belonging to the department of Sucre. According to the Territorial Planning Plande Orderingand the Instituto Geográfico Agustín Codazzi, this municipality is located in the Northeast of the department of Sucre, at 8º 55" and 9º 19" North latitude, and between 75º 25" and 74º 42" East of the Greenwich meridian.

The sampling was carried out in cattle farms established only with colosuana or kikuyina grass (Bothriochloa pertusa (L) A. Camus) in the municipality of Corozal, located according to the Territorial Planning Plande Orderingand the Instituto Geográfico Agustín Codazzi, (1998) in four agrological zones according to the classification of land by its capacity of use and management.

Sample collection

A representative sampling was carried out on the cattle farms, taking 15 to 20 random subsamples at a depth of 0-20 cm, collecting soil and roots at the same time. The subsamples were homogenized to form a sample per farm with an approximate weight of 2000 grams, which were deposited in plastic bags labelled with the farm number,townshiparea sown with grass and date of collection. A survey form was filled out for each farm sampled. The sample taken from each livestock farm was divided into two equal portions of 1 kg for physical-chemical and microbiological analysis.

Spore isolations. The samples were sieved to separate the coarse parts of the soil (stones, gravel) and roots. Once sieved, spores were isolated using the technique proposed by Daniels and Skipper, 1991; Sieverding, 1983 and Botero, 1998.

Spore counting. From each sample processed by the previous technique, two millilitres were taken and placed in the nematode counting chamber (advance equine products 5004-228th Avenue SI Issaquah, Washington 98029 USA) Menge and Timmer, where three counts per sample were carried out to obtain an estimate of the total number of spores in 100 grams of soil per farm (Perez el al-. 2022)

morphospeciesseparation

After each respective count, the contents of the chamber were collected in petri dishes, observed under a stereoscope and with the help of a dissecting needle morphotypes were collected, taking into account similarity of shape, color and size of the spores. With the help of a micropipette, the spores of the different morphotypes found were extracted, placed in test tubes with sterile water, labelled with the number of the farm where they were isolated and kept in a refrigerator at 4°C for 3 days, for subsequent identification and multiplication in the greenhouse.

Identification of genus

The isolated morphotypes were deposited in petri dishes, observed under a stereoscope to detail their characteristics in water, verify and eliminate spores of other morphospecies and contaminating particles. Once the spores were cleaned and the morphotype was verified, identification at the genus level was carried out, using techniques proposed by Schenck and Pérez, 1996, Morton and INVAM, 2003.

Percentage of infection

The determination of the percentage of infection in roots was done by methodologies proposed by Kormanik and McGraw (1991); and Botero (1998). Roots stained by this technique were placed parallel on slides, covered with slides and observed with a 40X objective, and 100 fields were counted in an orderly manner. Negative and positive fields were determined for each field. In the positive fields, the type of structure present (arbuscules, vesicles, cenocitic hyphae and spores) was taken into account (Sieverding 1983).

Multiplication of morphospecies

Of all the isolated morphotypes, those that appeared repeatedly and in greater proportion to the others were selected to be multiplied at greenhouse level with the grass species Brachiaria decumbens and three replicates, in the city of Sincelejo, using the pot culture technique proposed by Morton and Barea, (1996).

Evaluation of morphotypes. 120 days after inoculation of the morphotypes in the pot culture, soil samples were taken to count spores and the percentage of infection in roots. Based on these two criteria, the three best morphotypes were selected for field evaluation.

Field efficiency test. The field efficiency test was carried out by the farm, representative of the municipality of Corozal. Soil sampling was carried out for physical-chemical soil characterization and microbiological analysis. For this test, 60 plots of 0.5 x 0.5 m by 0.20 m depth were constructed, with 1.5 m separation between plots and 2 m between replicates. The soil of each plot was removed and chopped to provide a good seed bed, and the vegetative parts (rhizome and stolons) were removed to avoid regrowth.

Following the method used by Peña, (1999) the sterilization of the corresponding treatments was done by applying the fungicide Basamid G, in doses of 10 grams per plot (0.25 m2), the soil was mixed with the product to a depth of 0.2 m and irrigation was applied in order to distribute the fungicide in the soil. The plots were covered with black plastic for 8 days. After this time, the plots were uncovered, irrigation was applied and 10 g of sexual seeds of the grass species B. pertusa were sown. Ten days after germination and when the seedlings were 5 cm high, holes were made near the root system of the seedlings and inoculate (soil + spores + fragments of colonized roots) were applied, following Safir's guidelines. The inoculum used was mycorrhizal soil with spores and colonized roots. The determination of the amount of grams of mycorrhizal soil per individual morphotype and in mixture was made on the basis of 8000 spores per plot. For each individual morphotype, the number of g of soil was calculated to give a total of 8000 spores. For the treatments with a mixture of the three morphotypes, the amount of soil grams was determined individually using equal proportions until 8000 spores were obtained. During the experiment, temperature, rainfall and relative humidity were recorded.

Field trial evaluation

At 120 days after inoculation of the morphospecies, foliage samples were taken per treatment and repetition at a height of 10 cm from the soil surface, cutting and weighing the whole area of the plot to obtain forage production as a function of area. For forage production based on dry matter, samples per treatment and replicate were sent to the laboratory. For the determination of the nutritive value, samples were taken for each of the treatments. The nutritional content was obtained by determining the percentages of crude protein (CP), acid and neutral detergent fibre (FDA, NDF), fibre, ash and phosphorus. At 120 days, simultaneously with the samples for the determination of dry matter and nutritive value, a soil and root sample was taken for each replicate and spore counts and percentage of infection in roots were carried out individually for each treatment and replicate.

3. RESULTS AND DISCUSSION

Figure 1 shows the average spore density/100 g of soil and percentage (%) of infection in roots by agrological zones, which were obtained from the counts carried out on each farm. Zone 4 had the highest densities, while zone 1 had the lowest density. It is also observed that the highest percentage of Bothriochloa pertusa infection in roots was found in zone 1 and the lowest in zone 4. The percentage of root colonization was also observed, with a higher percentage of root colonization in cattle farms located in agroecological zone 1 and a lower percentage of root colonization in farms located in zone 4.

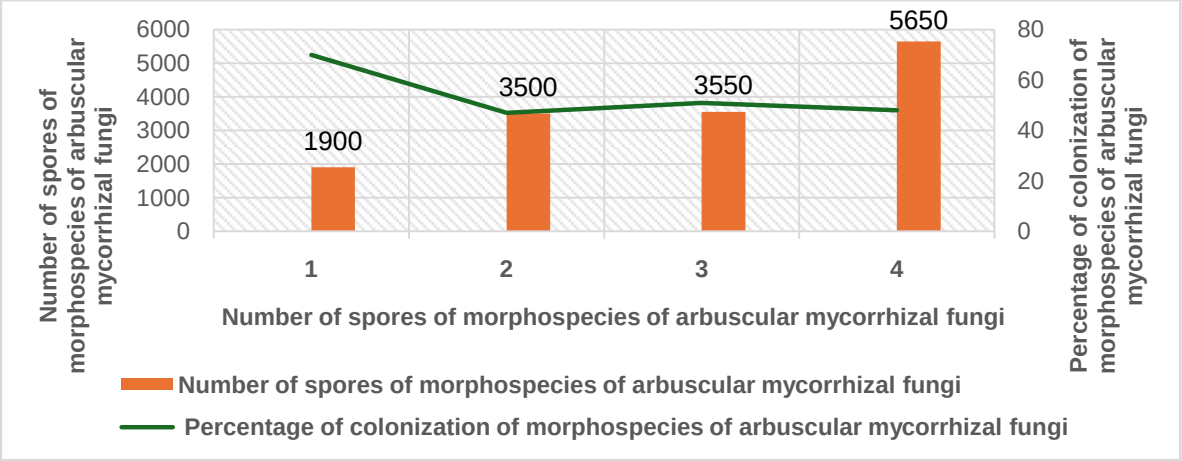

Figure 1. Spore density/100 g soil and percentage of infection in roots of B. pertusa by agrological zones.

Figure 1 shows that there is an inverse relationship between spore density and the percentage of infection found in this study, which is in agreement with that reported by Salamanca in his research carried out in the department of Guaviare with the grass species Brachiaria decumbens and Brachiaria dictyoneura.

The different degrees of compaction currently present in the soils of the different livestock farms do not seem to have had a marked influence on the abundance of spores and the infection percentages found. In this study, the high spore densities and infection percentages found in the agrological zones sampled were found in soils with low phosphorus and organic matter contents, which is similar to what was reported by Schultz, (1991) in his research on Andropogon gerardi, the most abundant grass species in North American prairies, who reports that this species is highly colonized by arbuscular mycorrhizae when grown in soils with low phosphorus content in the Kansas region, and also to that reported by Alloush in his studies, where he states that the high colonization of arbuscular mycorrhizae stimulates plant growth in soils with low fertility levels, especially P and M. O.

Figure 2 shows that the highest number of structures (spores, mycelia, vesicles and arbuscules) found in the root system of Colosuana grass during the determination of the percentage of infection occurred in zone 3, while zone 2 had the lowest formation of these structures. The greatest number of structures observed were hyphae, followed by vesicles and, to a lesser extent, arbuscules and spores.

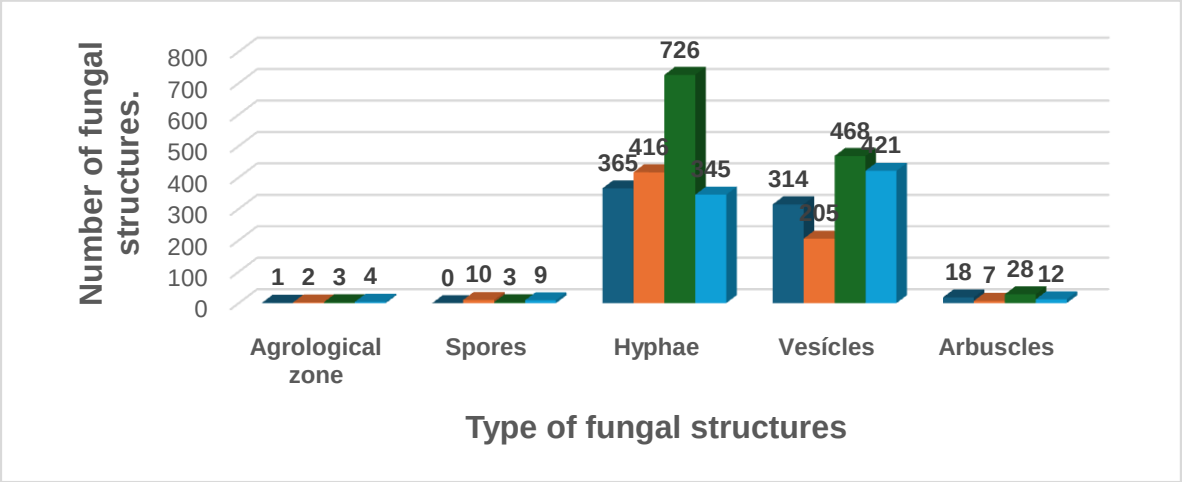

Figure 2. Number of fungal structures found in root tissue per sampled agroecological zone.

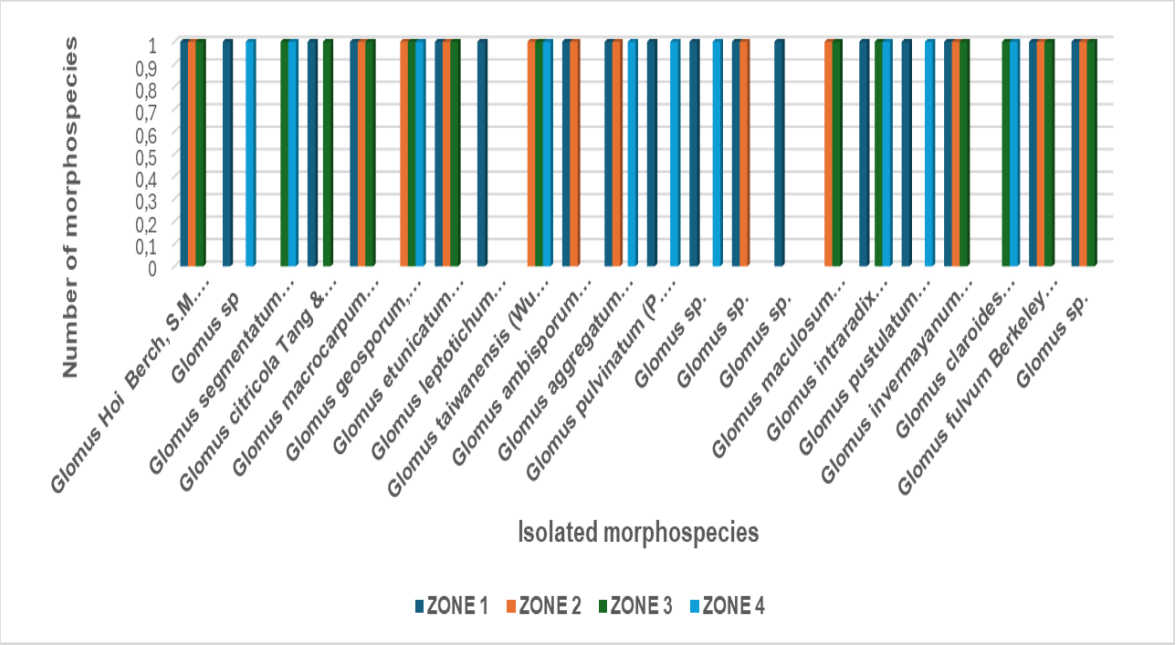

Figure 3. The number of morphospecies isolated on livestock farms located in four evaluated agroecological zones in the municipality of Corozal.

Figure 3 show that the highest number of isolated morphotypes occurred in agrological zone 1 and the lowest in zones 2 and 3.Figure 4, shows the behaviours of the morphospecies tested in the greenhouse with the grass species Brachiaria decumbens during 4 months, with average temperatures ranging from 27.5 - 32° C. Morphotype 19 presented the lowest values of spore density/100 g of soil and percentage of infection in roots, while morphospecies 5, 18 and 22 presented the highest spore densities, with morphospecies 22 reaching the highest percentage of infection in roots; which indicates that these morphospecies have a high capacity for multiplication and colonization on Brachiaria decumbens, allowing these results to select these three morphospecies for use in the field efficiency test with the grass B. pertusa (L.) A. Camus.

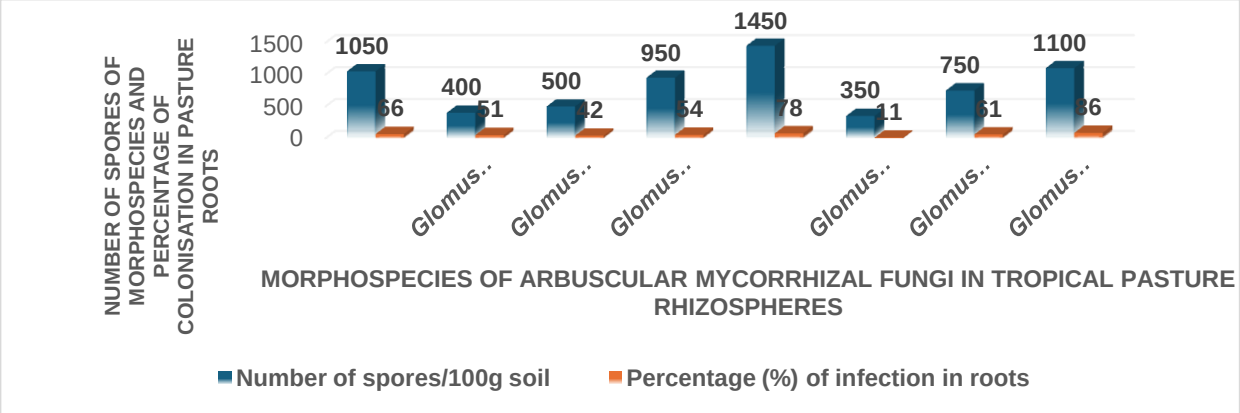

Figure 4. Spore amount and percentage of infection of morphospecies of bioaugmented mycorrhiza-forming fungi in trap culture with Brachiaria decumbens and use in field efficiency test.

The results obtained in this test are in agreement with those of Miranda, (1981), in his work carried out in Brazil with Brachiaria decumbens grass, who found that the percentage of infection and the number of spores of the genera Gigospora and Glomus is affected by the season, being higher in the rainy season. Similar results were found by Safir, (1996) in the Eastern Plains of Colombia on several plant species.

Field efficiency test. (Microbiological analysis)

Sampling yielded the following results: number of spores/100 g soil of 1700 like ranges found in zone 1. The percentage of infection was 38, results concordant with those obtained in agrological zone 2. Figure 5 shows the quantity in grams of mycorrhizal soil applied as inoculum source of morphospecies 5, 18 and 22 (individually and in mixture) in the field efficiency test for a total of 8000 spores per treatment.

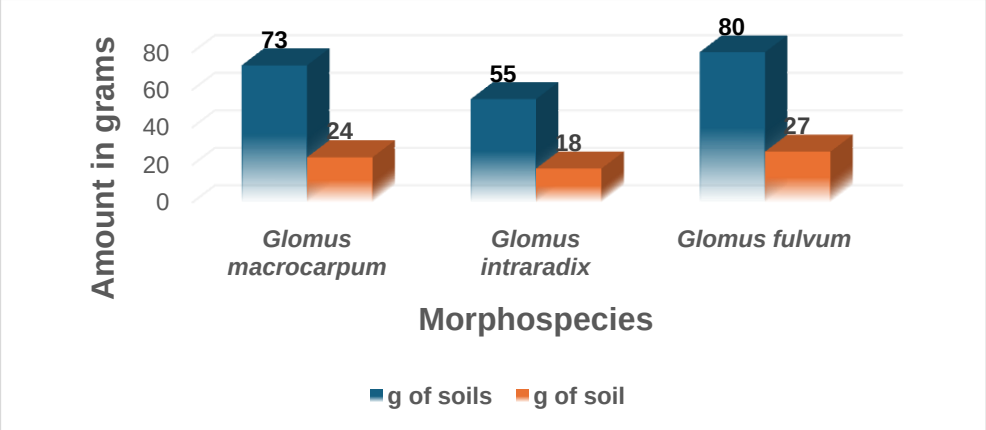

Figure 5. Morfoespecies y cantidad en g de suelo micorrizado usado en prueba de eficiencia en campo.

The results of the analysis of variance ($P < 0.01$) show that there was a highly significant difference between the different treatments in relation to the density of spores/100 g of soil. When averages were compared using Tukey's test ($P < 0.05$), a significant difference was found between treatments T8 and T9 with respect to T6 in relation to spore density. Treatment T6 presented significant differences with respect to treatments T1, T2, T3, T4 and T5; these treatments in turn were statistically equal to T7. All the above indicates that the highest spore density occurred in the treatment where sterile soil with morphospecies 22 was used, and the lowest was obtained in the negative control (T6), even below the natural control (T1) and the natural soil treatments with separate morphotypes and in mixture. It was also observed that spore density was similar in the treatment with sterile soil inoculated with morphospecies 18 in relation to the positive control and the natural soils inoculated with morphospecies.

When the analysis of variance ($P < 0.01$) was carried out, a highly significant difference was found between the treatments with respect to the percentage of infection in roots. The results of the comparison of averages by means of the Tuckey test ($P < 0.05$) show that there is a significant difference between treatments T10 and T6 in relation to the percentage of infection. Treatment T6 presented a significant difference with treatments T1, T2, T3, T4 and T5. It is also observed that treatments T3, T4 and T5 are statistically equal to each other and to treatments T7 and T9 with respect to the percentage of infection in roots.

The above indicates that the maximum percentage of infection in roots was obtained with the treatments where sterile soil was used with mixtures of morphospecies 5, 18 and 22 and with morphospecies 18. The lowest percentage of infection was presented in the negative control treatment. The treatments with natural soil inoculated with a mixture of morphotypes 5, 18 and 22 and with morphotypes 18 and 22 applied separately, showed similar percentages of root infections to the sterile soil treatments with morphotypes 5 and 22.

The analysis of variance ($P < 0.01$) was performed, highly significant differences were found between the different treatments with respect to forage production based on dry matter. When averages were compared by Tukey's test ($P < 0.05$) in relation to forage production based on dry matter kg/ha, there was a significant difference between treatments T10 and T6. Treatments T1 and T6 did not present significant difference, they are statistically equal in relation to forage production based on dry matter. Treatment T1 showed significant differences with treatments T3, T4 and T5. Treatment T5 showed significant differences with treatments T1 and T6 and was statistically equal to treatments T7, T8, T9. Treatments T2, T3, T4 and T5 are statistically equal to each other. This indicates that the highest forage production was obtained in the treatments where a mixture of morphotypes 5, 18 and 22 was applied in sterile soils, while the negative control treatment (sterile soil without morphotypes) presented the lowest yields, which was very similar to the natural control.

In addition, it was found that the treatments with natural soil inoculated with morphotypes 18 and 22 separately, and the mixture of morphotypes 5, 18 and 22, obtained higher forage production than the non-inoculated treatments. It is also observed that the T5 treatment with a mixture of the morphospecies presented a forage production like those of the treatments with separate morphospecies and in mixtures in sterilized soil. Table 1 shows that treatment T4 (natural soil + morphotype 22) had the highest percentage of ash, while treatment T6 (sterile soil without inoculation) had the lowest value.

The highest protein percentage was obtained in treatment T5 (natural soil + mixture of morphospecies 5, 18, 22) in relation to treatment T6 (sterile soil without inoculation), which presented the lowest value. On the other hand, it is observed that treatment T8 (sterile soil + morphospecies 18), presented the highest percentage of Neutral Detergent Fibre (NDF), and the minimum was registered in treatment T3 (natural soil + morphospecies 18). This indicates that morphotype 18 performed better in sterile soil than in natural soil in relation to the results obtained for NDF. The same table shows that the T9 treatment (sterile soil + morphospecies 22) presented the maximum percentage of fibre in acid detergent (FDA), while in the T4 treatment (natural soil + morphospecies 22) it obtained the minimum value. According to these results, it can be deduced that the efficiency of morphospecies 22 in sterile soil is higher than in natural soil. Regarding the percentage of phosphorus in plant tissues of B. pertus (L.) A. Camus, it was found that treatment T8 presented the highest value, while treatments T6 and T1 had the lowest percentages. Treatments T2, T3, T4, T5, T7 and T10 showed very similar values.

Table 1. Results of efficiency test with arbuscular mycorrhizal morphospecies on the nutritional status of pasture Bothriochloa pertusa (L). A. Camus.

Treatments	**Ashes (%)**	**Proteins (%)**	**FDN (%)**	**FDA (%)**	**P (%)**
T_1: (Natural Soil = SN)	7,19	2,46	74,25	41,6	0,079
T_2: (SN + Glomus macrocarpum)	8,6	2,39	75,04	45,25	0,11
T_3: (SN + Glomus pustulatum)	8,1	2,74	68,36	42,71	0,11
T_4: (SN + Glomus fulvum)	11,4	2,40	70,86	38,61	0,13
T_5: (SN + Glomus macrocarpum + Glomus pustulatum + Glomus fulvum)	7,18	2,99	74,75	44,07	0,11
T_6: (Sterile Soil = SE)	1,06	2,33	70,31	45,25	0,068
T_7: (SE + Glomus macrocarpum)	8,02	2,82	74,85	44,07	0,10
T_8: (SE + Glomus pustulatum)	7,76	2,58	75,78	42,20	0,90
T_9: (SE + Glomus fulvum)	7,48	2,62	75,24	46,42	0,09
T_{10}: (SE + Glomus macrocarpum + Glomus pustulatum + Glomus fulvum)	8,55	2,59	73,22	44,77	0,12

FDN: fibre in neutral detergent, FDA: fibre in acid detergent and P: phosphorus.Morphotype

All the above indicates that the treatments with natural and sterilized soils where arbuscular mycorrhizal fungi morphotype was inoculated, presented the highest concentration of phosphorus in the foliar tissues, which can be attributed to the action carried out by these microorganisms. In turn, treatments T1 and T6 corresponding to natural soil and sterilized soil without inoculation with arbuscular mycorrhizae, showed the lowest percentage of phosphorus in the natural tissues compared to the other treatments that received inoculation with morphotypes. Research carried out by Miranda 1981, with Brachiaria decumbens grass under greenhouse conditions, found an

increase in dry matter production and phosphorus uptake in plants harvested after 60 days, being three times higher in soils with arbuscular mycorrhizae than in non-mycorrhizal soils.

4. CONCLUSIONS

Thirty-one native morphospecies of arbuscular mycorrhizal fungi were isolated from cattle farms in the municipality of Corozal, established with Bothriochloa pertusa (L) A. Camus grass. Two genera were found: Glomus, predominantly species of the genus Glomus. These results contribute significantly to the knowledge of the biodiversity of beneficial soil microorganisms. The spore density/100 g of soil 2600 and infection percentages 41 in roots of Bothriochloa pertusa (L) A. Camus found in the municipality of Corozal were high. The highest spore density was found in zone 4 and the highest percentage of colonization in zone 1. The cattle farms under study, despite not receiving adequate agronomic management, show high spore densities and root colonization of Bothriochloa pertusa (L) A. Camus. The 8 morphospecies tested in the greenhouse showed different degrees of colonization on the grass species Brachiaria decumbens. Morphotypes 5, 18 and 22 showed the highest spore densities and percentages of colonization on roots. morphospecies s 5, 18 and 22 in the field research test showed statistically significant difference with respect to forage production on dry matter basis, when inoculated individually and in mixture on Bothriochloa pertusa (L) A. Camus in sterile soil. Similarly, they showed a similar behaviours when inoculated in mixture on natural soil. The percentages of ash, protein and phosphorus in Bothriochloa pertusa (L) A. Camus forage were higher in the inoculated treatments than in the non-inoculated treatments.

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6. Author contribution

Alexander Perez Cordero: experiment execution, data analysis.Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

7. Conflict of interest

All the authors of the manuscript declare that they have no conflict of interest.

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