Journal of Namibian Studies, 31 (2022): 191-204 ISSN: 2197-5523 (online)

Bioprospecting Of Arbuscular Mycorrhizae-Forming Fungi For Forage Production In Tropical Pastures

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ABSTRACT

The aim of this study was to evaluate the efficiency of native arbuscular mycorrhizal fungi associated with forage production in the grass species Bothriochloa pertusa (L) A. Camus in cattle 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 results of analysis of variance and Tukey's test show that the morphospecies (Glomus macrocarpum Tulasne & Tulasne, 1983; Glomus pustulatum Koske, Friese, Walker & Dalpe, 1986 and Glomus fulvum (Berkeley & Broome) Trappe & Gerdermann, 1922), corresponding to treatments T7, T8 and T9, respectively, tested in sterile soils, showed high efficiency in forage production in colosoana grass, high phosphorus concentration in plant tissues, and also showed high spore density and percentage of colonization in roots. These three morphospecies showed high efficiency in

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forage production and in improving the nutritional quality of colosoana grass in the field.

Key words. Microorganisms, soil, efficiency, forage, biomass, nutritional value.

1. INTRODUCTION

Among the introduced species, colosuana or kikuyina grass (Bothriochloa pertusa (L). A. Camus) represents the largest area planted 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 ha (Espitia and Pérez, 2016).

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 (Pérez et al., 2012). The sustainability of both natural ecosystems and agro-ecosystems is considered to depend primarily on the balance between the biological components of the soil. Indeed, it is accepted that the overall aim of the current and global trend in soil microbiology research is the study of microorganisms from the ecological, genetic, biochemical and physiological point of view in relation to plant nutrition and protection (Barea, 2002; Pérez et al., 2012).

The Arbuscular Mycorrhizal Fungi (AMF) are beneficial microorganisms that form symbiotic relationships with the roots of many terrestrial vascular plants as they are adapted to diverse environmental conditions (Tabassum et al., 2016; Caruso et al., 2018; Andersone-Ozola et al., 2021). This symbiotic relationship allows the host plants to boost their functioning by improving photosynthesis, stomatal conductance and osmolyte synthesis to uphold turgor pressure and the cellular functions needed for the process of metabolism under water-deficient conditions (Auge et al., 2016). Several studies reported that AMF improves the uptake of water and nutrients (particularly P) during their interaction with plant roots and subsequently enhances nodulation and N fixation of legumes even under P deficient soils (Arumugam et al., 2010; Bitterlich et al., 2018). Recently, they have been used in modern agriculture for promoting the uptake of mineral nutrients in the host plants, improving crop yield and quality while simultaneously reducing the use of chemical fertilizers, pesticides, and herbicides, as well as increasing plant resistance to salinity, heavy metals and drought stresses (Stepkowski et al., 2018; Golubkina et al., 2019, 2020; Jekabsone et al., 2022). The objective of this study was to isolate and evaluate the efficiency of arbuscular mycorrhizal fungi on the forage production of colosoana grass in the municipality of Corozal, department of Sucre, Colombia, with the aim of improving the nutritional value of this pasture.

2. MATERIALS AND METHODS

Spore isolation of arbuscular mycorrhizal fungi. Spore isolation of arbuscular mycorrhizal fungi from soil rhizosphere of cattle farms planted with colosoana grass in the municipality of Corozal, department of Sucre, Colombia, using the protocol proposed by Espitia and Pérez, (2016).

Determination of the percentage of colonization. The percentage of colonization of arbuscular mycorrhizal fungi was determined on stained roots using the technique proposed by Espitia and Pérez (2016).

Identification of morphospecies. The isolated morphospecies were deposited in Petri dishes, observed under a stereoscope to detail their characteristics in water, verify and eliminate spores of other morphotypes and contaminating particles. Once the spores were cleaned and verified, they were identified to genus and/or morphospecies level, using techniques proposed by Schenck and Pérez, (1990), Morton (1996), Oehl et al., (2011), INVAM (2021).

Field stage. This was carried out on cattle farms in the municipality of Corozal, Department of Sucre, Colombia.

Multiplication of morphotypes. From the isolated morphospecies, those with the highest prevalence and proportion with respect to the others were selected to be multiplied at greenhouse level with the grass species Brachiaria decumbens and three replicates, using the pot culture technique proposed by INVAM, 2021.

Evaluation of morphospecies. 120 days after the 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.

Experimental design of the field efficiency test. It was carried out using a completely randomized design with 6 replications and 10 treatments and with the following variables:

Independent variables

Evaluation of dry matter and green forage production.

Analysis of nutritional content (crude protein, fiber in acid and neutral detergent).

Dependent variables. Constituted by three morphospecies (Glomus macrocarpum,
(Tulasne & Tulasne, 1983); Glomus pustulatum Koske, Friese, Walker & Dalpe,
1986 and Glomus fulvum (Berkeley & Broome) Trappe & Gerdermann, 1922)
selected for their greater capacity of sporulation and infection in roots, which were
distributed in 10 treatments at random, as follows:

Treatments:

T1: Natural soil (positive control, without morphospecies) + Bothriochloa pertusa (L). A. Camus

T2: Natural soil + Bothriochloa pertusa (L). A. Camus + morphospecies 1 (Glomus macrocarpum, Tulasne & Tulasne, 1983)

T3: Natural soil + Bothriochloa pertusa (L). A. Camus + morphospecies 2 (Glomus pustulatum Koske, Friese, Walker & Dalpe, 1986)

T4: Natural soil + Bothriochloa pertusa (L). A. Camus + morphospecies 3 (Glomus fulvum (Berkeley & Broome) Trappe & Gerdermann, 1922)

T5: Natural soil + Bothriochloa pertusa (L). A. Camus + morphospecies consortium 1, 2 and 3

T6: Sterile soil + Bothriochloa pertusa (L). A. Camus (negative control, no morphospecies)

T7: Sterile soil + Bothriochloa pertusa (L). A. Camus + morphospecies 1

T8: Sterile soil + Bothriochloa pertusa (L). A. Camus + morphospecies 2

T9: Sterile soil + Bothriochloa pertusa (L). A. Camus + morphospecies 3

T10: Sterile soil + Bothriochloa pertusa (L). A. Camus + morphospecies consortium 1, 2, 3.

Field experiment. The field efficiency test was carried out on a representative livestock farm in the municipality of Corozal. Soil sampling was carried out for physico-chemical characterization of the soil and microbiological analysis using the methodologies. 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 repetitions. 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 prevent regrowth.

The sterilization of the plots was carried out using the methodology proposed by Peña. 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 with the purpose of distributing 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 Botriochloa 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 inocula (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 with individual and mixed morphospecies in the amount of 8000 spores per plot.

For each individual morphospecies, the number of grams 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. Temperature, rainfall and relative humidity were recorded during the experiment.

Evaluation of the experiment

Forage and dry matter production. At 120 days after inoculation of the morphospecies, foliage samples were taken per treatment and replicate 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 on a dry matter basis, samples per treatment and replicate were sent to specialized laboratories for determination. 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 fiber (FDA, NDF), fiber, ash and phosphorus.

Spore count and determination of the percentage of colonization. After 120 days, simultaneously with the samples for the determination of dry matter and nutritive value, a sample of soil and roots was taken per replicate and spore count and percentage of infection in roots was carried out individually for each treatment and replicate, using the protocol proposed by Espitia and Pérez, (2016).

3. RESULTS AND DISCUSSION

The three morphospecies (Glomus macrocarpum Tulasne & Tulasne, 1983; Glomus pustulatum Koske, Friese, Walker & Dalpe, 1986 and Glomus fulvum (Berkeley & Broome) Trappe & Gerdermann, 1922) were selected for their higher sporulation and infection capacity in roots. Figure 1 shows the morphological characteristics in 40X light microscopy (figure 1). **Physical-chemical and microbiological evaluation**. Prior to the establishment of the efficiency test in the field, a physical-chemical analysis of the soil and a microbiological analysis were carried out, with the following results.

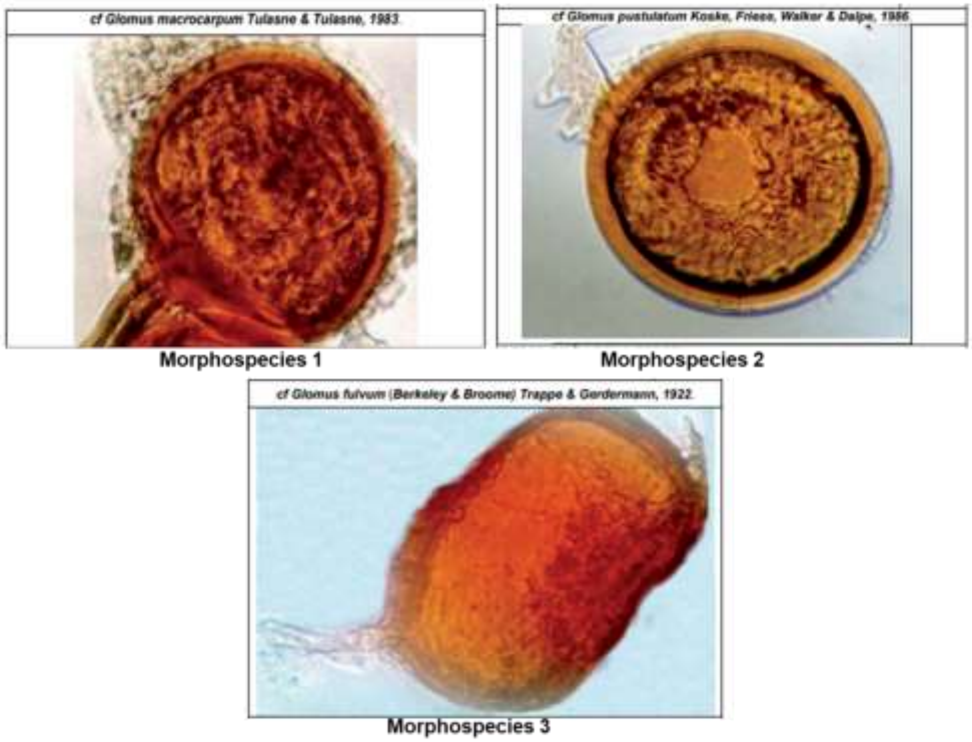

Figure 1. Morphospecies of arbuscular mycorrhizal fungi evaluated in a field efficiency test with colosoana grass in the municipality of Corozal, Sucre-Colombia.

- **Physical-chemical analysis**. Table 1 shows the results of the physical-chemical analysis, its physical and chemical components of the soil where the field test was carried out.

Table 1. Numerical descriptive statistics (mean ± standard deviation), analysis of variance and Tukey's test of chemical parameters of livestock farm soils used for field trial.

Variables	Locality (mean)
pH	5,44 ± 0,2
Organic matter	1,23 ± 0,89
Nitrogen	0,0012 ± 0,0008
Phosphorus	3,11 ± 3,80
Cation exchange capacity	22,13 ± 4,92
Sodium	0,50 ± 0,45
Potassium	0,51 ± 0,51
Calcium	11,32 ± 3,12
Magnesium	6,47 ± 5,75

The results of the analysis of variance (Table 1) for chemical parameters of the soil in livestock soils of the municipality of Corozal, indicate that there were significant differences between the values of pH, Phosphorus and Sodium of the soil of the livestock farms analyzed in relation to the other chemical parameters evaluated. Normality was not obtained for the variables Nitrogen, Phosphorus, Sodium, Potassium, analysis of variance (ANOVA) was carried out by the non-parametric method of Kruskal-Wallis and separation of means by ranges by the Tukey procedure.

- **Microbiological analysis.** Sampling yielded the following results: the number of spores/100 g of soil was (1600) 1700 (-1800) and the percentage of infection was (36-) 38 (-40%).
- **Inocula used in the field test.** The inocula (treatments) with the morphospecies as described in table 2. In the table, the amount in grams of mycorrhizal soils, applied as source of inoculums, of morphospecies 1, 2 and 3 (individually and in consortium) in the field efficiency test to obtain a total of 8000 spores per treatment is recorded.

Table 2. Amount of mycorrhizal soil in (g) used in field efficiency test.

Morphospecies	g of Soil (individual)	g of Soil (consortion 1:3)
Glomus macrocarpum Tulasne & Tulasne, 1983	73	24
Glomus pustulatum Koske, Friese, Walker & Dalpe,1986	55	18
Glomus fulvum (Berkeley & Broome) Trappe & Gerdermann, 1922	79,5	26,5

Spore density. 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 was used with the morphospecies Glomus fulvum (Berkeley & Broome) Trappe & Gerdermann, 1922 and the lowest was obtained in the negative control (T6), even below the natural control (T1) and the natural soil treatments with morphospecies alone and in consortia. It was also observed that the spore density was similar in the treatment with sterile soil inoculated with the morphospecies Glomus pustulatum Koske, Friese, Walker & Dalpe, 1986 in relation to the positive control and the natural soils inoculated with the morphospecies used.

Percentage of infection in roots. When analysis of variance ($P < 0.01$) was carried out, a highly significant difference was found between 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.

This indicates that the highest percentage of infection in roots was obtained with the treatments where sterile soil was used with consortium with the morphospecies used and with morphospecies 2. The lowest percentage of infection was in the negative control treatment. The treatments with natural soil inoculated with the three morphospecies in consortium and with morphotypes 2 and 3 applied separately, showed similar percentages of root infections as the treatments with sterile soil with morphospecies 1 and 3.

Evaluation of forage production. When 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 difference with treatments T3, T4 and T5. Treatment T5 showed significant difference 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 the consortium with the three morphospecies was applied in sterile soils, while the negative control treatment (sterile soil without morphospecies) 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 morphospecies 2 and 3 separately, and the consortium with the three morphospecies, presented higher forage production than the non-inoculated treatments. It was also observed that the T5 treatment with a mixture of the morphotypes presented a forage production similar to those of the treatments with separate morphotypes and in mixtures in sterilized soil.

Figure 2 shows that treatment T4 (natural soil + morphospecies 3) had the highest percentage of ash, while treatment T6 (sterile soil without inoculation) had the lowest value.

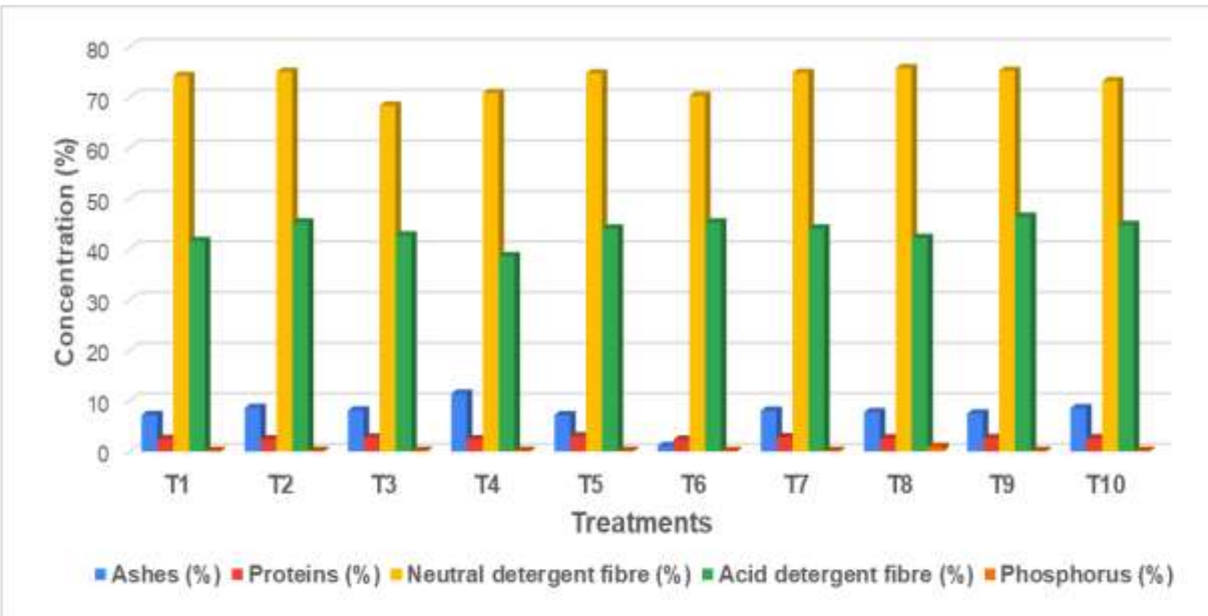

Figure 2. Nutritional content analysis of Bothriochloa pertusa (L). A. Camus 120 days after the field experiment was established.

The highest protein percentage was obtained in treatment T5 (natural soil + consortium with morphospecies) 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 2), presented the highest percentage of Neutral Detergent Fiber (NDF), and the minimum was registered in treatment

T3 (natural soil + morphospecies 2). This indicates that it performed better in sterile soil than in natural soil in relation to the results obtained for NDF.

In the same table it can be seen that treatment T9 (sterile soil + morphospecies 3) presented the maximum percentage of Acid Detergent Fiber (FDA), while in treatment T4 (natural soil + morphospecies 3) it obtained the minimum value. According to these results, it can be deduced that the efficiency of morphospecies 3 (Glomus fulvum (Berkeley & Broome) Trappe & Gerdermann, 1922) in sterile soil is higher than in natural soil.

With regard to 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 presented very similar values.

All of the above indicates that the treatments with natural and sterilized soils where morphospecies of arbuscular mycorrhizal fungi were inoculated presented the highest concentration of phosphorus in the leaf 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 arbuscular mycorrhizae inoculation, showed the lowest percentage of phosphorus in the natural tissues compared to the other treatments that received inoculation with Glomus pustulatum morphotypes Koske, Friese, Walker & Dalpe, 1986. Results of experimental work 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. CONCLUSION

The morphospecies of arbuscular mycorrhizal fungi tested in the greenhouse showed different degrees of colonization on the grass species Brachiaria decumbens. The morphospecies identified as: morphospecies (Glomus macrocarpum Tulasne & Tulasne, 1983; Glomus pustulatum Koske, Friese, Walker & Dalpe, 1986 and Glomus fulvum (Berkeley & Broome) Trappe & Gerdermann, 1922) showed the highest spore densities and colonization rates on roots.

The three morphospecies evaluated 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 behaviour when inoculated in consortium in natural soil.

The percentages of ash, protein and phosphorus in Bothriochloa pertusa (L) A. Camus forage were higher in the treatments that received inoculation with respect to the treatments not inoculated with arbuscular mycorrhiza-forming fungi.

5. 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.

6. CONFLICT OF INTEREST. All the authors of the manuscript declare that they have no conflict of interest.

7. ACKNOWLEDGEMENTS

The authors would like to thank the Microbiological Research Laboratory of the University of Sucre for their support in the development of this research activity.

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