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Use of Mycorrhizal Fungi to Increase the Production of Three Tropical Legumes with Forage Potential

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Abstract:

The present study has the objective to use mycorrhizal fungi to improve the production of dry matter in three legumes which are considered as forage use. The design used was randomized complete blocks. Treatments evaluated were mycorrhizal fungi, Fertilized inorganically, Commercial biofertilizer and Control without inoculum. The variable evaluated was dry matter production in three periods of evaluation. The highest values of dry matter production observed in *Mucuna pruriens* were 196.5g/$m^2$for the control, 194.5 g/m^2mycorrhiza treatmentand 192.4 g/$m^2$for inoculated treatment, in respect to *Clitoriaternatea*, control and mycorrhiza treatments showed the best yielding with 182.3 and 163.1 g/m^2 respectively and for *Centrosemamacrocarpum*were the treatments not fertilized and mycorrhiza with 25.4, 23.5 and 20.8 g/m^2 respectively. Our results showed that mycorrhizal could be an option to fertilize these legumes which could be include in livestock production, on the other hand, mycorrhizal in soils can be capable to associate with many plant forage species.

Keywords **—biofertilizers, livestock, forage species, organic agriculture.**

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I. INTRODUCTION

The quality of forage species is usually limited by good nutrition in soils (Ford *et al*., 2019), furthermore, in order to improve the dry matter production is necessary to use high quantity of inorganic nutrients (Martin, 2018). In tropical regions, adequate quantity of nitrogen is not available for plants in soils (Figueroa *et al*., 2020) furthermore that the costs are higher (Khadda, 2021; Obando *et al*., 2010). One of the solutions to resolve this problem is the use of legumes forage species that have the capacity to obtain great quantity of nutrients and are nutritive source for ruminants (Castro-Montoya and Dickhoefer, 2020; Gaviria-Uribe *et al*., 2020), furthermore, those legumes have the potential to reduce the CO_2 emitted during the manufacturing of chemical nitrogenous fertilizers through their biological nitrogen fixation (BNF) capacity (Romanyà and Casals, 2020; Kumar *et al*., 2018). Another characteristic of those legumes forage species is the symbiosis with certain microorganisms to obtain better nutrients.

The use of this type of microorganisms in agriculture is called biofertilization which can be an alternative to reduce the use of inorganic fertilizers in forage production (Itelima, 2018). The use of this products offers an economic and ecologic possibility for farmers (Bhowmik and Das, 2018). The beneficial microorganisms for plants usually interact in the rhizosphere (Mishra *et al*., 2017)

which is an area when occurs a wide biological and chemical processes that provide maintenance, operation and stability of agricultural production systems (Aguirre, 2006), uptake of nutrients for the plant as well as nitrogen fixation (Hu *et al*., 2021), transport and solubilization of phosphorus (Stamenković*et al*., 2018), production of growing regulatory substances in roots etc. (Aguirre, 2006).

The use of biofertilizers is a technology in agroecosystems that can help to generate microhabitats in rhizosphere (Patel *et al*., 2021),moreover, they can contribute to reduce the use of inorganic fertilizers (Divan *et al*., 2008; Mayz-Figueroa, 2004; FAO y GTIS, 2015).

One of the biofertilizer that is used in nitrogen fixation is the mycorrhizal. Those microorganisms are a symbiotic mutualistic relationship between special soil fungi and fine plant root system. Mycorrhizal fungi are a heterogeneous group of diverse fungal taxa, associated with the roots of over 90% of all plant species (Nath and Meena, 2018). In this symbiotic mutualistic, fungus supplies inorganic nutrients to the plant and plant supplies organic nutrients. The establishment of this association implies strengths interdependences between fungi and plant to the point that the mycorrhiza is part of the root system (Corredor, 2008). Mycorrhizal association are generated in all vascular plant with some exceptions as Crucifers, Quenopodiaceae, Ciperaceae, Caryophylaceae and Juncaceae families (Azcón Aguilar y Barea, 1997).

Although mycorrhizal associations are generally considered non-specific, there could be specific compatibility between certain fungus-plant species. On the other hand, they present broad scientific interest due to their usefulness in studies of a physiological nature in plants, in microbiology, applications to biotechnology in commercial horticultural and ornamental production and in the recovery of arid zones and degraded soils (Hernández and Chialloux, 2001). It can also be used against rhizosphere pathogens. Accord the above mentioned the objective of this research is to use mycorrhizal fungi to improve the production of three tropical legumes with forage potential.

II. MATERIALS AND METHODS

Study area, soil and climate conditions

The present study was carried out in laboratory of Instituto Nacional de InvestigacionesForestales, Agrícolas y Pecuarias (INIFAP), campo experimental Chetumal and plot of co-worker farmer from Xul-Ha that is located in Othon P. Blanco, Quintana Roo at 3.5 km of Xul-Ha community with 21°30' N and 89°29' W coordinates at 10 masl. Climate conditions are 27.6° C and 62.3% of relative humidity on average, annual medium precipitation is 1300 mm and the period with the most precipitation was from June to November with 70% of precipitation. The soils of the study area are the so-called chromic luvisols, characterized by having a good content of organic matter.

Obtention of samples from rhizosphere

It was selected five better plants of the next species: *Clitoriaternatea*, *Mucuna pruriens*, *Leucaena leucocephala*, *Sesbania sesban*, *Pueraria phaseoloides*, *Centrosemapubescens*, *Centrosemasp*, *Cajanus cajan* and*Macroptilliumatropurpureum*. Then, it was used the Hoffer barrier to obtain five samples per plant from rhizosphere. In total it was obtained 25 samples per species which were mixed to homogenize and then taken 500 g per species. The samples were collected in plastic bags to get in the laboratory.

Isolation of Mycorrhizal fungi

To isolate the mycorrhizal fungi, it was used the sieved and decanted technique by Ferrera-Cerrato (1993). In the first step, it was carried out a suspension with 100 g of manure dissolved in 2 liters of water, and then the suspension was shaken in mechanical way for five minutes and rested three minutes in order to eliminate big particles by sedimentation. The suspension was filtered and washed with enough water in 500 and 44 μM sieves. This process was carried out twice. The final filtering was put on graph paper and counted in stereoscope microscopy to be classified.

Inoculant reproduction based from mycorrhizal fungi

For reproducing these fungi, it was considered the type of host plant. In this case, it was selected a plant with strong dependence with mycorrhizal fungi. These plants were short cycle plants (from 4 to 6 months) with abundant radical system. When plant life cycle finished, the aerial parts of the plant were separated and roots were dried in the same substrate at room temperature. Then, the colonized rootlets with mycelium and spores were extracted and hammer mill crushed. The inoculum was pun inside of plastic bags and cooled at 4°C. Finally, it was prepared a suspension with inoculum and was applied directly in plants.

Biofertilizers evaluation in field and experimental design

For field evaluations were used *Mucuna pruriens*, *Clitoriaternatea*, and *Centrosemamacrocarpum* as host plants. The species were sown in a row in rows of five meters. The size of the plot was for each species four rows of 5 m each, at distances of 0.5 m from each other. The sampling area covered the two central rows up to 0.25 m on each side of them and not including the final 0.5 m at each end (of its length). The total dimensions of the effective or sample plot were 1 m x 4 m = 4 m^2. The total sampling plot was divided into four subplots of 1 m^2 each for independent samplings taken after regrowth, for this purpose the frame was used. Three repetitions per treatment were sown. The design used was randomized complete blocks. Treatments evaluated were mycorrhizal fungi, fertilized inorganically, commercial biofertilizer and control without inoculum. The variable evaluated was dry matter production in three periods of evaluation. An analysis of variance and Tuckey's test were performed for the difference of means.

III. RESULTS AND DISCUSSION

Identification of mycorrhizal fungi

The isolation of mycorrhizal fungi allowed the identification of three spores of black, amber and brown colorwhich depended on the type of soil and host plant. The types of mycorrhiza observed were arbuscular of the genus *Glomus*, which is characterized by forming intracellular arbuscules and undoubtedly those of greater diffusion and economic and ecological importance due to the symbiotic association that they form with the host plants. Mycorrhizal associations are generally considered non-specific, that is, any symbiont fungus can colonize any receptive plant (Sanders, 2002). However, there may be a preference or better compatibility affinity between certain fungus-plant pairs (Genre *et al*., 2020; Tedersoo*et al*., 2020). In contrast, there are also cases such as Eucalyptus in which the total associative non-specificity means that these and other species are colonized at the same time by formations as different as *ectomycorrhizae* and *endomycorrhizae* (Sugiyama and Sato, 2021; Clasen*et al*., 2018).

Dry matter production in treatments with mycorrhizal fungi in field

Dry matter production (DM) in treatments evaluated in *Mucuna pruriens*showed differences between treatments ($P \leq 0.05$). The highest values without finding a significant difference between them were 196.5, 194.5 and 192.4 g/m^2 of DM for the control, mycorrhiza and inoculated with Rhizobium treatments respectively, compared with the fertilized and commercial ones that presented a production of 173.7 and 178.4 g/m^2 of DM respectively (Table 1). Biofertilizers applied to grass and legume meadows have shown their benefits in very short periods (Aguirre, 2006), as can be seen in these data obtained for *M. pruriens*, since although the control treatment had the highest value, no one was found. High significance compared to biofertilizer treatments.

Table 1. Yield of dry matter by treatments for *Mucuna pruriens*

Treatments	Yielding of dry matter (g/m^2)			
	1st period	2nd period	3th period	Average
Mycorrhizal fungi	160.8^a	240.4^a	182.4^c	194.5a
Fertilized inorganically	136.3^c	166^d	218.7^a	173.7b
Commercial biofertilizer	130.2^c	215.3^c	189.8^c	178.4b
Control without inoculum	147.4^b	238.7^b	203.5^b	196.5a

Similar letters correspond to treatments statistically equal accord Tukey test (P≤0.05).

In respect with DM production in *Clitoriaternatea*, it was observed that the behavior of this legume varies according to the treatments since the maximum values have significant differences, and correspond to the control and mycorrhiza treatments with yields of 182.3 and 163.1 g/m^2 respectively. These results agree with that reported by Aguirre (2006) who observed in a study with legumes that treatments inoculated with the microorganisms alone or in combination showed similar trends in terms of the accumulation of dry matter compared to the control without inoculation. This response could be to the plants that are in native soils have the capacity to create easy symbiosis with different microorganisms and produce DM accord environment plant requirements (Wang*et al*., 2021).

Table 2. Yield of dry matter by treatments for *Clitoriaternatea*

Treatments	Yielding of dry matter (g/m^2)			
	1st period	2nd period	3th period	Average
Mycorrhizal fungi	81.5^b	348^a	60^d	163.1^b
Fertilized inorganically	58.2^c	296^b	89^c	147.7^c
Commercial biofertilizer	80^b	179^d	99^c	119.3^d
Control without inoculum	148^a	244^c	155^a	182.3^a

Similar letters correspond to treatments statistically equal accord Tukey test (P≤0.05).

For *Centrosemamacrocarpum*, significant differences (P≤0.05) were observed between the treatments not fertilized and mycorrhiza for dry matter production with 25.4, 23.5 and 20.8 g/m^{2}respectively.The fertilizer and commercial biofertilizer treatments presented DM production of 1.0 and 2.92 g/m^2 respectively. This response could be that the application of high doses of nitrogen have adverse effects on the various crops (Timsina, 2018; Rizvi and Khan, 2018; Coskun*et al*., 2017).When the availability of nitrogen is high in the soil, the microorganisms do not fulfill their function of fixing atmospheric nitrogen (Jones*et al*., 2018) but take up the available nitrogen in the soil, therefore the symbiotic process is not established (Masson-Boivin and Sachs, 2018).

Table 3. Yield of dry matter by treatments for *Centrosemamacrocarpum*

Treatments	Yielding of dry matter (g/m2)			
	1st period	2nd period	3th period	Average
Mycorrhizal fungi	4^b	12.8^a	53.8^b	23.5^a
Fertilized inorganically	1^d	1^c	1^d	1.00^c
Commercial biofertilizer	2.7^c	2^c	4^c	2.92^c
Control without inoculum	5^b	6.6^b	64.6^a	25.4^a

Similar letters correspond to treatments statistically equal accord Tukey test (P≤0.05).

IV. CONCLUSIONS

Mhycorrizal in soils can be capable to associate with many plant forage species. The host plants *Mucuna pruriens, Clitoriaternatea*and *Centrosemamacrocarpum* could be alternative

species to be used in livestock due to their capacity to associate with mycorrhizal fungi, furthermore, these findings propose solutions to use this species legumes with biofertilizers in tropical livestock production.

REFERENCES

1) Aguirre, J. F. (2006). Biofertilizantes microbianos; su aplicación en la agricultura. Tuxtla chico, Chiapas, México. Centro de Investigación Regional Pacifico Sur. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Rosario Izapa. (Folleto Técnico N° 1). 21 p.
2) Azcón-Aguilar, C. and Barea, J.M. (1996). Arbuscular mycorrhizas and biological control of soil-borne plant pathogens and overview of the mechanisms involve. Mycorrhiza, 6, 457-464.
3) Bhowmik, S. N., and Das, A. (2018). Biofertilizers: a sustainable approach for pulse production. In Legumes for Soil Health and Sustainable Management (pp. 445-485). Springer, Singapore.
4) Campbell, D.H., Garvey, F.S., Cremer, N.E. and Sussdort, D.H. (1970). In: W.A. Benjamin (ed.). Methods in immunology. 2nd ed. MacMillan. New York. 435-436 p.
5) Castro-Montoya, J. M., and Dickhoefer, U. (2020). The nutritional value of tropical legume forages fed to ruminants as affected by their growth habit and fed form: A systematic review. Animal FeedScience and Technology, 114641.
6) CIAT. (1988). Simbiosis leguminosa-rizobio; manual de métodos de evaluación, selección y manejo agronómico. Ed. Rev. Proyecto CIAT-UNDP de evaluación, selección y manejo de la simbiosis leguminosa-rizobio para aumentar la fijación de nitrógeno.
7) Clasen, B. E., Silveira, A. D. O., Baldoni, D. B., Montagner, D. F., Jacques, R. J. S., and Antoniolli, Z. I. (2018). Characterization of ectomycorrhizal species through molecular biology tools and morphotyping. ScientiaAgricola, 75, 246-254.
8) Coskun, D., Britto, D. T., Shi, W., and Kronzucker, H. J. (2017). Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. NaturePlants, 3(6), 1-10.
9) Divan, V., Silva, J., dos Santos, K., Baldani, J., and Massena, V. (2008). Inoculants base on nitrogen-fixing bactéria*Azospirillum* spp. and their application in tropical agriculture. Asociación Argentina de Microbiología, BA, 227–237.
10) FAO, and GTIS. (2015). Estado mundial del recurso suelo. Resumen Técnico. (L. Caon, N. Forlano, M. Keene, A. Sorokin, I. Verbeke, *and* C. Ward, Eds.). Roma: FAO.
11) Ferrera-Cerrato. R. (1993). Manual de Agromicrobiologìa. Ed. Trillas. Mèxico. 144 p.
12) Figueroa, D., Ortega-Fernández, P., Abbruzzini, T. F., Rivero-Villlar, A., Galindo, F., Chavez-Vergara, B. and Campo, J. (2020). Effects of Land Use Change from Natural Forest to Livestock on Soil C, N and P Dynamics along a Rainfall Gradient in Mexico. Sustainability, 12(20), 8656.
13) Ford, M. M., Zamora, D. S., Current, D., Magner, J., Wyatt, G., Walter, W. D., and Vaughan, S. (2019). Impact of managed woodland grazing on forage quantity, quality and livestock performance: the potential for silvopasture in Central Minnesota, USA. Agroforestrysystems, 93(1), 67-79.
14) Gaviria-Uribe, X., Bolivar, D. M., Rosenstock, T. S., Molina-Botero, I. C., Chirinda, N., Barahona, R., and Arango, J. (2020). Nutritional quality, voluntary intake and enteric methane emissions of diets based on novel Cayman grass and its associations with two Leucaena shrub legumes. Frontiers in VeterinaryScience, 7, 764.
15) Genre, A., Lanfranco, L., Perotto, S., and Bonfante, P. (2020). Unique and common traits in mycorrhizal symbioses. NatureReviewsMicrobiology, 18(11), 649-660.
16) Hernández, María I. and Chialloux, Marisa. 2001. La nutrición mineral y la biofertilización en el cultivo del tomate (*Licopersiconesculentum* Mill). Temas de Ciencia y Tecnología, 5(13),11
17) Hu, H. Y., Li, H., Hao, M. M., Ren, Y. N., Zhang, M. K., Liu, R. Y. and Kuzyakov, Y. (2021). Nitrogen fixation and crop productivity enhancements co - driven by intercrop root exudates and key rhizosphere bacteria. Journal ofAppliedEcology, 58(10), 2243-2255.
18) Itelima, J. U., Bang, W. J., Onyimba, I. A., Sila, M. D., and Egbere, O. J. (2018). Bio-fertilizers as key player in enhancing soil fertility and crop productivity: A review.
19) Jones, D. L., Magthab, E. A., Gleeson, D. B., Hill, P. W., Sánchez-Rodríguez, A. R., Roberts, P. and Murphy, D. V. (2018). Microbial competition for nitrogen and carbon is as intense in the subsoil as in the topsoil. SoilBiology and Biochemistry, 117, 72-82.
20) Khadda, B. S. (2021). Prospects of organic farming in India. A VoiceforAgriculture, 27.
21) Kumar, S., Meena, R. S., Lal, R., Yadav, G. S., Mitran, T., Meena, B. L. and Ayman, E. S. (2018). Role of legumes in soil carbon sequestration. In Legumes for Soil Health and Sustainable Management (pp. 109-138). Springer, Singapore.
22) Martin, K. E. (2018). Effects of nitrogen fertilizer and regrowth interval on herbage dry matter yield, chemical composition and nitrogen solubility of alternative pasture forages in irrigated Canterbury conditions (Doctoral dissertation, Lincoln University).
23) Masson-Boivin, C., and Sachs, J. L. (2018). Symbiotic nitrogen fixation by rhizobia—the roots of a success story. CurrentOpinion in Plant Biology, 44, 7-15.
24) Mayz-Figueroa, J. (2004). Fijación biológica de nitrógeno. Revista Científica UDO Agrícola, 4(1), 1–20. Retrievedfrom https://dialnet.unirioja.es/servlet/ articulo?codigo=2221548.
25) Mishra, J., Singh, R. and Arora, N. K. (2017). Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. Frontiers in microbiology, 8, 1706.
26) Nath, D., and Meena, V. S. (2018). Mycorrhizae: A potential microorganism and its implication in agriculture. In Role of rhizospheric microbes in soil (pp. 251-276). Springer, Singapore.
27) Obando, D., Burgos, L., Rivera, D., Rubiano, M., Bonilla, R., *and*Divan, V. (2010). Caracterización de bacterias diazotróficasasimbióticas asociadas al eucalipto (eucalyptussp.) en codazzi, cesar. Acta Biológica Colombiana, 15(3), 107–120.
28) Patel, N., Butani, N., and Desai, P. (2021). Functional Diversity in Rhizosphere Microbial Community: Concept to Applications. In MicrobialMetatranscriptomicsBelowground (pp. 343-365). Springer, Singapore.
29) Rizvi, A., and Khan, M. S. (2018). Heavy metal induced oxidative damage and root morphology alterations of maize (*Zea mays* L.) plants and stress mitigation by metal tolerant nitrogen fixing Azotobacter chroococcum. Ecotoxicology and environmental safety, 157, 9-20.
30) Romanyà, J., and Casals, P. (2020). Biological nitrogen fixation response to soil fertility is species-dependent in annual legumes. Journal ofSoilScience and Plant Nutrition, 20(2), 546-556.
31) Sanders, I. R. (2002). Specificity in the arbuscular mycorrhizal symbiosis. In Mycorrhizalecology (pp. 415-437). Springer, Berlin, Heidelberg.
32) Stamenković, S., Beškoski, V., Karabegović, I., Lazić, M., and Nikolić, N. (2018). Microbial fertilizers: A comprehensive review of current findings and future perspectives. Spanish Journal ofAgriculturalResearch, 16(1).
33) Sugiyama, Y., and Sato, H. (2021). The Limited Establishment of Native Ectomycorrhizal Fungi in Exotic Eucalyptus spp. Stands in Japan. Frontiers in microbiology, 12, 376.
34) Tedersoo, L., Bahram, M., and Zobel, M. (2020). How mycorrhizal associations drive plant population and community biology. Science, 367(6480).
35) Timsina, J. (2018). Can organic sources of nutrients increase crop yields to meet global food demand?. Agronomy, 8(10), 214.
36) Wang, X., Feng, H., Wang, Y., Wang, M., Xie, X., Chang, H. and Wang, E. (2021). Mycorrhizal symbiosis modulates the rhizosphere microbiota to promote rhizobia–legume symbiosis. Molecular Plant, 14(3), 503-51

