Scientific Paper

Biofertilization with *Azospirillum brasilense* and *Rhizoglomus irregulare* in *Tithonia diversifolia* (Hemsl.)

Sergio Méndez-Bonet[1] https://orcid.org/0000-0002-0679-7802, Pedro José González-Cañizares[2] https://orcid.org/0000-0003-3206-0609, Reynerio Reyes-Rouseaux[3] https://orcid.org/0000-0001-7526-8456, Juan Francisco Ramírez-Pedroso[3] https://orcid.org/0000-0003-3384-3904

[1]*Universidad Agraria de la Habana Fructuoso Rodríguez Pérez. Carrera Tapaste y Autopista Nacional km 23 1/2, San José de las Lajas, Mayabeque, Cuba. CP: 32700, Apartado Postal: 18-19.* [2]*Instituto Nacional de Ciencias Agrícolas. Gaveta Postal No. 1. San José de las Lajas, Mayabeque. Cuba.* [3]*Instituto de Investigaciones de Pastos y Forrajes. Avenida Independencia, km 8 ½ Boyeros, La Habana, Cuba. E-mail:* sergio92@unah.edu.cu, pgonzalez@inca.edu.cu, ramirez@pastos.vcl.minag.cu

Abstract

Objective: To evaluate the effect of simple and combined biofertilization of *Azospirillum brasilense* and *Rhizoglomus irregulare* on the nutritional status and biomass yield of *Tithonia diversifolia* (Hemsl.).

Materials and Methods: In a field experiment four treatments were studied, made up by the simple and combined inoculation of *A. brasilense* and *R. irregulare* plus a non-inoculated control, in a Latin square design. The inoculant with *R. irregulare*, with a concentration of 30 spores g^{-1}, was applied through the use of *Canavalia ensiformis* (L.) DC as preceding crop and a way for the reproduction of mycorrhizal fungi in the soil. *A. brasilense*, with a concentration of 10^9 CFU mL^{-1}, was administered at a rate of 20 L ha^{-1}, 15 days after planting. The frequency and intensity of the mycorhizal colonization, the concentrations of NPK in the biomass of the aerial part and dry matter yields, were evaluated.

Results: *R. irregulare* increased the frequency and intensity of mycorrhizal colonization, as well as the number of spores in the rhizosphere. Nevertheless, the highest effects were reached when it was applied jointly with *A. brasilense*. The biofertilizers separately increased the nitrogen concentrations in the aerial biomass and the dry matter yields, but the highest values of these variables, as well as the highest efficiency of biofertilization, were obtained with the joint application of both. The effect of biofertilization remained during the year in which the experiment was conducted.

Conclusions: The simple inoculation with *A. brasilense* and *R. irregulare* improves the nitrogen nutrition and biomass yields of *T. diversifolia*. With the joint application of both biofertilizers, higher effectiveness of the mycorrhizal inoculation is achieved. In addition, higher values are achieved in these indicators compared with the results reached with each one separately.

Keywords: *Azospirillum brasilense*, growth, nutritional status, fungi, yield

Introduction

Tithonia diversifolia (Hemsl.), commonly known as Mexican sunflower, is a plant of the family *Asteraceae*. Originated in Central America, it adapts well to tropical region and is cultivated in diverse countries as forage resource, due to its high biomass production, fast recovery after being cut and nutritional value (Cerdas-Ramírez, 2018).

This species extracts from the soil important quantities of nutrients for biomass production (Botero-Londoño *et al*., 2019), so that when it is cultivated as forage, it needs adequate fertilization to maintain its productivity and preserve fertility of the edaphic medium (López-Guzmán *et al*., 2017; Santos *et al*., 2021). However, the high prices of fertilizers and the need to promote an environment-friendly agriculture, suggest the search for fertilization strategies that guarantee adequate nutrition of the crops, decrease the use of external inputs and, in turn, ensure the protection of natural resources (Finkel *et al*., 2017). In the last years much attention has been paid to the use of biofertilizers, because of their direct effect on the improvement of the biological properties of the soils, increase of crop yields and reduction of the use of synthetic fertilizers (Agarwal *et al*., 2018).

Among the microorganisms used as biofertilizers the associative bacterium *Azospirillum brasilense* is found, capable of fixing atmospheric nitrogen and improving the crop productivity and quality, besides other benefits for the production of phytohormones, solubilization of phosphates and protection of plants against abiotic stresses (Fukami *et al*., 2018; Oliveira *et al*., 2018). In addition, the biofertilization with arbuscular mycorrhizae-forming fungi, which increase the soil volume, explore the roots and

Received: 09/08/2021
Accepted: 23/12/2021

How to cite a paper: Méndez-Bonet, Sergio; González-Cañizares, Pedro José; Reyes-Rouseaux, Reynerio & Ramírez-Pedroso, Juan Francisco. Biofertilization with *Azospirillum brasilense* and *Rhizoglomus irregulare* in *Tithonia diversifolia* (Hemsl.).. *Pastos y Forrajes*. 45:eI8, 2022.

facilitate the absorption of nutrients and water, has shown its effectiveness in different crops (Chandrasekaran, 2020; Simó-González *et al.,* 2020). However, few works approach the effect of the utilization of both biofertilizers on the forage production of *T. diversifolia*.

From these premises, the objective of this study was to evaluate the effect of simple and combined biofertilization of *Azospirillum brasilense* and *Rhizoglomus irregulare,* on the nutritional status and biomass yield of *T. diversifolia*.

Materials and Methods

Location. The experiment was conducted in a dairy farm of the Basic Unit of Cooperative Production (UBPC, for its initials in Spanish) Juan Oramas, located in the Guanabacoa municipality, Havana province, at 23°08' North latitude and 82°11' West longitude.

Soil. The study was carried out on a carbonated smooth brown soil (Hernández-Jiménez *et al*., 2015), whose main chemical characteristics are shown in table 1.

For the soil analysis the following methods were applied: pH in H_2O (potentiometry, soil-water ratio 1:2.5), organic matter content (Walkley and Black), assimilable P (extraction with H_2SO_4 0.5 mol L^{-1} and colorimetric determination), exchangeable bases (extraction with NH_4Ac 1 mol L^{-1} pH 7, determination by complexometry for Ca and Mg and flame photometry for Na and K) and base exchange capacity (sum of exchangeable bases), according to the analytical techniques established in the National Institute of Agricultural Sciences (INCA, for its initials in Spanish), described by Paneque *et al*. (2011).

Climate conditions. The performance of rainfall during the period in which the experiment was conducted (June, 2018-July, 2019) is shown in figure 1.

Treatment and experimental design. Four treatments were evaluated (biofertilization with *A. brasilense*, biofertilization with *R. irregulare*, biofertilization with *A. brasilense* + *R. irregulare* and a non-inoculated control), distributed in a Latin square to avoid possible variations in soil fertility and in the resident populations of arbuscular mycorrhizal fungi (AMF), originated by the spatial distribution and agronomic management of the plants previously cultivated in the area where the experiment was carried out, and which could increase the experimental error. Each treatment was distributed only once, in rows and columns. The plots constituted the experimental unit, with a total surface of 24 m^2 and a calculation area of 16 m^2.

Experimental procedure. The soil was prepared through plowing (plow), harrow, crossing (plow) and harrow, at approximate intervals of 25 days between each labor. Afterwards, it was plowed, and in May, 2018, all the plots were sown with *Canavalia ensiformis* (L.) DC, but it was inoculated with *R. irregulare* at the moment of planting in the two treatments corresponding to biofertilization with AMF, as a way to reproduce in the soil sufficient quantities of mycorrhizal propagules of *R. irregulare* for the later inoculation of *T. diversifolia* (Rivera *et al*., 2020).

C. ensiformis was sown at 50 cm between rows and 20 cm between plants. In the treatments with AMF, the inoculation was carried out by the method of seed covering. For such purpose a fluid paste was prepared by mixing solid inoculant and water, in proportion 1: 0.6 m v, where the seeds were submerged. They were dried under shade and immediately planted. The inoculant contained 30 spores per gram of the strain INCAM-11, of the AMF species *R. irregulare* (Sieverding *et al*., 2014), from the collection of the INCA.

In turn, *C. ensiformis* was cut at 60 days and its aerial biomass was removed from the field for feeding the animals. Table 2 shows the quantity of AMF spores that remained in the soil in the plots corresponding to each treatment, after cutting *C. ensiformis* and at the moment of planting *T. diversifolia*.

After cutting *C. ensiformis*, the soil was furrowed and *T. diversifolia* was sown, which was

Table 1. Chemical characteristics of the soil (depth 0-20 cm).

pH H_2O	OM %	P	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	BEC
		mg kg^{-1}	cmol kg^{-1}				
7,6	4,49	125	51,3	5,5	0,22	0,91	57,93
(0,1)	(0,20)	(18)	(2,4)	(0,9)	(0,09)	(0,12)	(3,75)

OM: organic matter, BEC: base exchange capacity, Values between parentheses indicate confidence intervals (α=0,05)

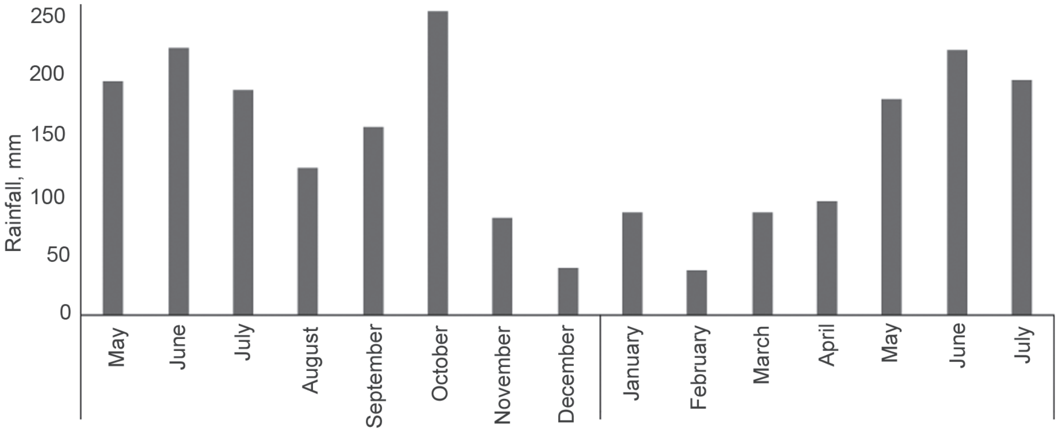

Figure 1. Performance of rainfall during the execution of the experiment
Source: Data taken in the experimental area

done through vegetative propagules of approximately 30 cm of length, taken from the top and medium part of the stems of a field cultivated with this species, very close to the experimental area. The propagules were planted at 1,0 m between rows and 0,5 m between plants.

At the moment of planting *T. diversifolia*, in each plot composite soil samples were taken, at a depth of 0-20 cm, on which pH in H_2O and the contents of organic matter, assimilable P, exchangeable bases and determination of Ca and Mg, Na and K, and base exchange capacity, were determined.

For the biofertilization with *A. brasilense* the commercial product Nitrofix® was used, from the Cuban Research Institute of Sugarcane Derivatives (ICIDCA, for its initials in Spanish), which contained the strain 8I, with a concentration of 10^9 CFU mL^{-1}. A mixture of liquid inoculant and water was prepared in 1:10 v / v ratio. Through a manual knapsack, 20 L ha^{-1} of the commercial product were applied to the soil. It was administered very close to the rows, 15 days after the sprouting of *T. diversifolia*. The experiment was conducted without irrigation and no basal fertilization was applied.

Measurements. Four cuts were performed. The first one was done 120 days after planting (November, 2018) and the others in February, May and July, 2019, at a height of 30 cm from the soil surface. In each cut the fresh biomass of the aerial part corresponding to the calculation area of the plots was weighed. Samples of 200 g were taken, which were carried to an air circulation stove at 70 ºC during 72 h, to calculate the dry matter percentage (DM) and estimate the DM yield. In the second and fourth cut, the N, P, K concentrations (g kg^{-1} DM) in the biomass were determined (Paneque *et al*., 2011).

At the moment of the second and fourth cut, framed in the rainy and dry seasons, respectively, three subsamples of roots and soil of the rizosphere of each plot were taken, at depth of 0-20 cm. For such purpose, a metallic cylinder of 5 cm diameter and 20 cm of height was used. The sampling points were distributed equidistantly and separated by 10 cm from the rows.

The subsamples were homogenized to form a composite sample per plot and 1 g of rootlets was extracted for its staining and clarification (Rodríguez-Yon *et al*., 2015). The frequency of mycorrhizal colonization through the method of intercepts (Giovanetti and Mosse, 1980) and the visual density or colonization intensity, according to Trouvelot *et al*. (1986), were evaluated, as well as the number of spores in the rizosphere from the wet-sieving and decanting of such structures and their observation in the microscope (Herrera *et al*., 1995).

The efficiency index (EI) of biofertilization was determined by the following formula:

EI: [Accumulated DM yield (t ha^{-1}) of the biofertilized treatment – Accumulated DM yield (t ha^{-1}) of the control treatment / Yield of the cumulative DM (t ha^{-1}) of the control treatment] x 100

Mathematical analysis. The data, once the variance normality and homogeneity were tested, were processed through variance analysis and Tukey's test ($p < 0.05$). In the variables corresponding to the chemical characteristics of the soil and the number of AMF spores that remained in the soil after cutting *C. ensiformis* (tables 1 and 2), as well

Table 2. Quantities of spores that remained in the soil after cutting *C. ensiformis*.

Treatment	Number of spores (50 g^{-1} of soil)
Control	87 (± 10)
R. intraradices	322 (± 15)
A. brasilense	93 (± 17)
R. intraradices + A. brasilense	331 (± 9)

Values between parentheses indicate confidence intervals (α=0,05)

as in those whose results are shown in the figures, the confidence interval was used (**α**=0,05) as dispersal stadigraph or as criterion for their comparison (Payton *et al*., 2000). In all cases the statistical program SPSS 25 (2017) was used.

Results and Discussion

The inoculation with *R. irregulare* increased the colonization frequency and intensity and the number of spores in the rhizosphere (fig. 2), which proved the effectiveness of this strain to colonize the roots of *T. diversifolia*. Nevertheless, the highest values were reached with the joint application of both biofertilizers. This performance could be ascribed to the increase of the colonization sites of the AMF from the positive effect exerted by the phytohormones produced by *A. brasilense* on the increase of root length and branching and, consequently, on the formation and multiplication of the mycorrhizal structures (Raklami *et al*., 2019).

The effect of *R. irregulare* was observed even in the fourth cut (July, 2019). That is, 12 months after planting *T. diversifolia*. Even the values of mycorrhizal colonization frequency, intensity and number of spores in the rhizosphere in this cut were higher than those recorded in the second one (February, 2019). This performance could be related to the seasons in which the sampling was conducted. During the rainy season a fast growth of crops occurs, due to the increase of rainfall volumes, temperature and luminosity. This implies the absorption of higher quantity of nutrients for biomass formation and, consequently, the formation of higher quantities of mycorhizal structures to guarantee the access of plants to the soil resources (Bhardwaj and Chandra, 2018).

The increase of the fungal variables in the treatments inoculated with *R. irregulare* also confirms the report by Rivera *et al*. (2020) about the effectiveness of the use of *C. ensiformis* as preceding crop and as a way for the mycorrhizal inoculation of the later crop, which implies an important reduction of the inoculant quantities to be used.

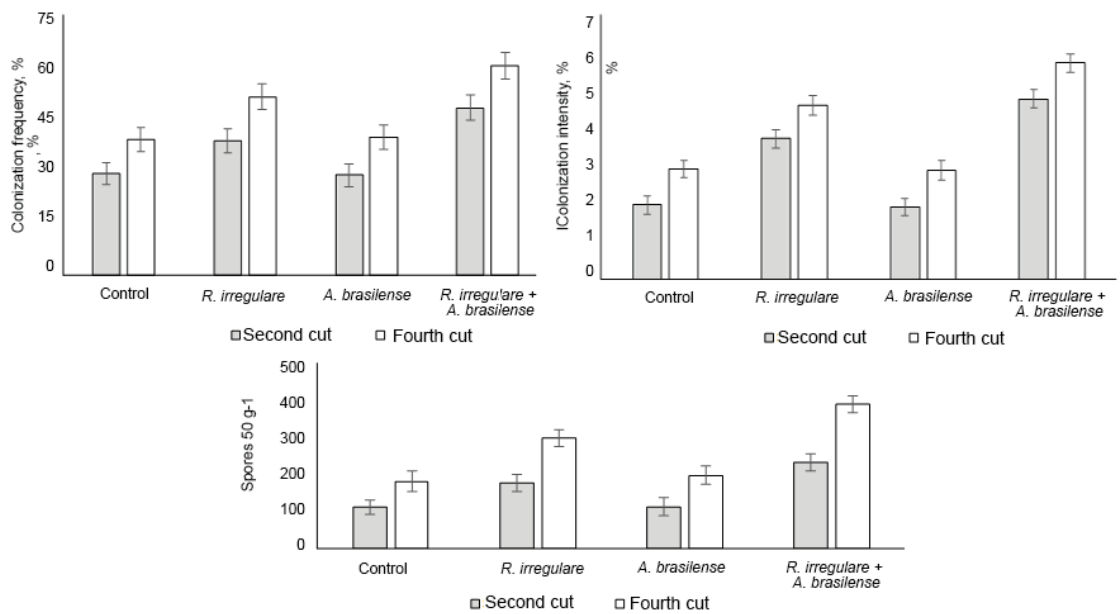

Figura 2. Effect of the treatments on the frequency and intensity of the mycorrhizal colonization and on the number of spores in the rhizosphere of *T. diversifolia*.

The vertical bars show the confidence interval. Means with confidence intervals that overlap do not differ significantly (α= 0,05).

According to the inoculation method used, in which a quantity of inoculant equivalent to 10 % of the seed or propagule weight, in *T. diversifolia* would imply the utilization of 100 kg ha^{-1} of inoculant. However, with the utilization of previously-inoculated *C. ensiformis*, as a way for the inoculation of *T. diversifolia* from the mycorrhizal propagules left by this crop on the soil, the quantity of inoculant was reduced to 6 kg ha^{-1}.

Table 3 shows the effect of biofertilization on the N, P and K concentrations in the biomass of the aerial part, in the second and fourth cut. *R. irregulare* and *A. brasilense* increased the N concentrations with regards to the non-inoculated treatment, although no significant differences were detected between both treatments. Yet, the highest effects were obtained with the joint application of the biofertilizers. No significant differences were found between the treatments for the N, P and K concentrations in the biomass. This performance was in correspondence with the high contents of both nutrients in the soil (Paneque and Calaña, 2001).

The dry matter yields had a similar performance to the one observed in the concentrations of nutrients in the biomass (table 4). That is, with the separate application of one or the other biofertilizer there was significant increase in this indicator, and both showed similar values. Nevertheless, the highest effect was obtained through the co-inoculation with both microorganisms.

The effect of biofertilizers on the increase of *T. diversifolia* yields seems to be related to their influence on the increase of N concentrations in the biomass. It is known that this crop absorbs important quantities of N (Santos *et al*., 2021), and judging by the organic matter content of the soil where the experiment was conducted, which is classified as moderate (Paneque and Calaña, 2001), the availability of this element in the soil might not have been sufficient to satisfy the crop demand. Thus, the biofertilizers could have contributed to satisfy these needs and, consequently, to improve the biomass yields.

When analyzing the efficiency index of biofertilization, which shows in percentage increase the biomass yields of each inoculated treatment with regards to the non-inoculated control, there were no differences between the separate application of one and the other biofertilizer. However, the highest value was obtained with the joint application of both (fig. 3).

AMF actively participate in the transference of N and other soil nutrients to the host plant through the mycelium network that increases the absorption

Table 3. Effect of biofertilizers on the N, P and K concentrations in the biomass (g kg^{-1}).

Treatment	Second cut			Fourth cut		
	N	P	K	N	P	K
Control	26,7^{c}	2,5	19,2	23,2 c	2,0	17,5
R. irregulare	32,1b	2,2	20,3	28,9 b	2,2	18,3
A. brasilense	31,7b	2,4	19,3	29,1 b	2,2	17,7
R. irregulare + *A. brasilense*	36,8 a	2,3	20,4	35,4 a	2,1	18,8
SE ±	0,501	0,213	0,416	0,419	0,107	0,311
P - value	0,002	0,347	0,256	0,001	0,301	0,415

Different letters in the same column significantly differ, according to Tukey's test (p < 0,05)

Table 4. Effect of the biofertilizers on the biomass yield of *T. diversifolia* (t dry matter ha^{-1}).

Treatment	First cut (November, 2018)	Second cut (February, 2019)	Third cut (May, 2019)	Fourth cut (July, 2019)
Control	3,7^{c}	3,5^{c}	3,3^{c}	5,9^{c}
R. irregulare	4,4^{b}	4,2^{b}	4,0^{b}	6,6^{b}
A. brasilense	4,4^{b}	4,3^{b}	4,0^{b}	6,7^{b}
R. irregulare + *A. brasilense*	5,1^{a}	5,0^{a}	4,6^{a}	7,5^{a}
SE ±	0,181	0,162	0,153	0,204
P - value	0,002	0,001	0,002	0,001

Averages with different letters in the same column significantly differ, according to Tukey's test (p < 0,05).

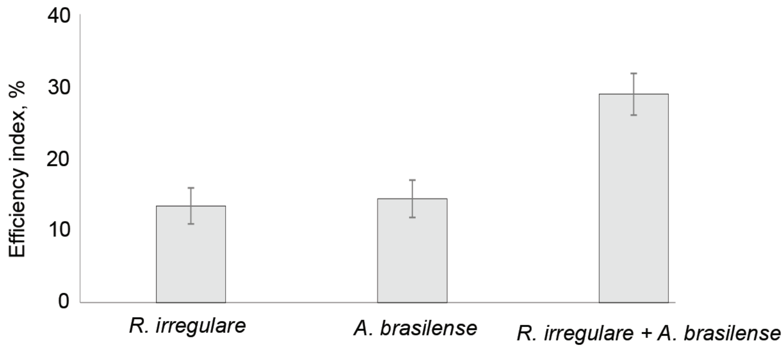

Fig. 3. Efficiency index of the biofertilization. Means with confidence intervals that overlap do not differ significantly (α= 0.05).

capacity of the roots of plants (Yu *et al*., 2020). *A. brasilense* can fix important quantities of atmospheric N and transfer it to the host plant (Aguirre *et al*., 2018). This is translated into an increase of yield, even, into a reduction of the needs of this nutrient, via fertilization (Leite *et al.,* 2019). But the fact that the highest effects have been obtained with the joint application of both biofertilizers proves that their benefits are complemented, which brings about a higher agronomic response than with the application of one or the other separately.

Siddaram *et al.* (2017) and Kamali and Mehraban (2020), when evaluating the effects of the co-inoculation with *A. brasilense* and AMF observed a tripartite interaction between both microorganisms and the host plant, whose synergic relations caused increase in the concentrations of nutrients and in plant growth, significantly higher than those reached with the application of each microorganism separately.

Another interesting aspect was the permanence of the effect of biofertilizers on the soil-plant system, which was observed even twelve months after planting *T. dversifolia*. This suggests that the benefits of biofertilization could be extended beyond that period, and suggests conducting studies in this regard.

It is concluded that the single inoculation with *A. brasilense* and *R. irregulare* improves the nitrogen nutrition and biomass yields of *Tithonia diversifolia*. With the joint application of both fertilizers, higher effectiveness of the mycorrhizal inoculation and values of these higher indicators area are achieved than those reached with each one separately. To study further about the co-inoculation with *A. brasilense* and *R. irregulare* and its contribution to the reduction of the use of biofertilizers is suggested.

Acknowledgements

The authors thank the Science and Innovation Financial Fund (FONCI, for its initials in Spanish) for the contribution to funding the execution of this work. Gratitude is also expressed to the M. Sc. Ana N. San Juan Rodríguez and Daisy Dopico Ramírez, specialists of the Cuban Sugarcane Derivative Research Institute, for donating the biofertilizer Nitrofix® ***for the execution of the experiment.***

Conflict of interests

The authors declare that there are no conflicts of interests among them.

Authors' contribution

- Sergio Méndez-Bonet. Participated in the execution of the experiment that led to the elaboration of the paper. Participated in the statistical processing and interpretation of the experimental results and in the paper writing.
- Pedro José González-Cañizares. Led the research task that allowed the elaboration of the paper, participated in the design of the experiment and interpretation of the results.
- Reynerio Reyes-Rouseaux. Participated in the execution of the experiment that led to the elaboration of the paper and participated in the interpretation of the results.
- Juan Francisco Ramírez-Pedroso. Participated in the execution of the experiment that led to the elaboration of the paper. Participated in the design and interpretation of the results of the experiment.

Biblographic references

Agarwal, P.; Gupta, Ritika & Gill, Imneet Kaur. Importance of biofertilizers in agriculture biotechnology. *Ann. Biol. Res.* 9 (3):1-3. https://www.

scholarsresearchlibrary.com/articles/importance-of-biofertilizers-in-agriculture-biotechnology-15125.html, 2018.

Aguirre, Priscila F.; Olivo, C. J.; Rodrigues, Patricia F.; Falk, Débora R.; Adams, Carine B. & Schiafino, Helena P. Forage yield of Coastcross-1 pastures inoculated with *Azospirillum brasilense*. *Acta Sci., Anim. Sci*. 40:e36392, 2018. DOI: https://doi.org/10.4025/actascianimsci.v40i1.36392.

Bhardwaj, A. K. & Chandra, K. K. Soil moisture fluctuation influences AMF root colonization and spore population in tree species planted in degraded entisol soil. *Int. J. Biosci*. 13 (3):229-243, 2018. DOI: http://dx.doi.org/10.12692/ijb/13.3.229-243.

Botero-Londoño, J. M.; Gómez-Carabalí, A. & Botero-Londoño, Mónica A. Yield, agronomic parameters and nutritional quality of *Tithonia diversifolia* in response to different fertilization levels. *Rev. mex. de cienc. pecuarias*. 10 (3):789-800, 2019. DOI: https://doi.org/10.22319/rmcp.v10i3.4667.

Cerdas-Ramírez, R. Extracción de nutrientes y productividad del botón de oro (*Tithonia diversifolia*) con varias dosis fertilización nitrogenada. *InterSedes*. 19 (39):172-187, 2018. DOI: https://doi.org/10.15517/isucr.v19i39.34076.

Chandrasekaran, A. A meta-analytical approach on arbuscular mycorrhizal fungi inoculation efficiency on plant growth and nutrient uptake. *Agriculture*. 10:370, 2020. DOI: https://doi.org/10.3390/agriculture10090370.

Finkel, O. M.; Castrillo, G.; Herrera-Paredes, S.; Salas-González, I. & Dangl, J. L. Understanding and exploiting plant beneficial microbes. *Curr. Opin. Plant Biol*. 38:155-163, 2017. DOI: https://doi.org/10.1016/j.pbi.2017.04.018.

Fukami, Josiane; Cerezini, Paula & Hungria, Mariangela. *Azospirillum*: benefits that go far beyond biological nitrogen fixation. *AMB Expr*. 8:73, 2018. DOI: https://doi.org/10.1186/s13568-018-0608-1.

Giovanetti, M. & Mosse, B. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol*. 84:489-500, 1980. DOI: https://doi.org/10.1111/j.1469-8137.1980.tb04556.x.

Hernández-Jiménez, A.; Pérez-Jiménez, J. M.; Bosch-Infante, D. & Castro-Speck, N. *Clasificación de los suelos de Cuba 2015*. Mayabeque, Cuba: Instituto Nacional de Ciencias Agrícolas, Instituto de Suelos, Ediciones INCA, 2015.

Herrera, A.; Ferrer, L.; Furrazola, E. & Orozco, M. O. Estrategia de funcionamiento de las micorrizas VA en un bosque tropical. Biodiversidad en Iberoamérica. Ecosistemas, evolución y procesos sociales. En: M. Monasterio, ed. Programa *Iberoamericano de Ciencia y Tecnología para el Desarrollo. Subprograma XII, Diversidad Biológica*. Mérida, México, 1995.

Kamali, S. & Mehraban, A. Effects of Nitroxin and arbuscular mycorrhizal fungi on the agro-physiological traits and grain yield of sorghum (*Sorghum bicolor* L.) under drought stress conditions. *PlosOne*. 15 (12):e0243824, 2020. DOI: https://doi.org/10.1371/journal.pone.0243824.

Leite, R. da C.; Oliveira, D. dos S. de; Santos, A. C. dos; Leite, R. da C. & Hungria, Mariangela. Mitigation of Mombasa grass (*Megathyrsus maximus*) dependence on nitrogen fertilización as a function of inoculation wiht *Azospirillum brasilense*. *Rev. Bras. Cienc. Solo*. 43:e0180234, 2019. DOI: https://doi.org/10.1590/18069657rbcs20180234.

López-Guzmán, Á. E.; Díaz-Jarquín, L. J. & Calero-Borge, W. A. Efecto de tres fertilizantes orgánicos en el crecimiento de botón de oro en condiciones de vivero, Nueva Guinea, RACCS. *Cienc. Intercult*. 24 (1):203-214, 2017. DOI: https://doi.org/10.5377/rci.v24i01.8016.

Oliveira, I.; Fontes, J. R. A.; Pereira, B. F. F. & Muñiz, A. W. Inoculation with *Azospirillum brasiliense* increases maize yield. *Chem. Biol. Technol. Agric*. 5:6, 2018. DOI: https://doi.org/10.1186/s40538-018-0118-z.

Paneque, V. M. & Calaña, J. M. *La fertilización de los cultivos. Aspectos teórico prácticos para su recomendación*. San José de las Lajas, Cuba: Instituto Nacional de Ciencias Agrícolas. p. 28, 2001.

Paneque, V. M.; Calaña, J. M.; Calderón, M.; Borges, Y.; Hernández, T. & Caruncho, M. *Manual de técnicas analíticas para análisis de suelo, foliar, abonos orgánicos y fertilizantes químicos*. Mayabeque, Cuba: Ediciones INCA, Instituto Nacional de Ciencias Agrícolas, 2011.

Payton, M. E.; Miller, A. E. & Raun, W. R. Testing statistical hypotheses using standard error bars and confidence intervals. *Commun. Soil Sci. Plant Anal*. 31 (5-6):547-551, 2000. DOI: https://doi.org/10.1080/00103620009370458.

Raklami, A.; Bechtaoui, N.; Tahiri, A.; Anli, M.; Meddich, A. & Oufdou, K. Use of rhizobacteria and mycorrhizae consortium in the open field as a strategy for improving crop nutrition, roductivity and soil fertility. *Front. Microbiol*. 10:1106, 2019. DOI: https://doi.org/10.3389/fmicb.2019.01106.

Rivera, R.; Martín, Gloria; Simó, J.; Pentón, Gertrudis; García-Rubido, Milagros; Ramírez, J. *et al*. Benefits of joint management of green manure and mycorrhizal inoculants in crop production. *Trop. Subtrop. Agroecosystems*. 23:97. https://www.revista.ccba.uady.mx/ojs/index.php/TSA/article/view/3294/1497, 2020.

Rodríguez-Yon, Yakelín; Arias-Pérez, Lianne; Medina-Carmona, Aida; Mujica-Pérez, Yonaisy; Medina-García, Laura R.; Fernández-Suárez, Kalyanne *et al.* Alternativa de la técnica de tinción para determinar la colonización micorrízica. *Cult. Trop.* 36 (2):18-21. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0258-59362015000200003&lng=es&tlng=es, 2015.

Santos, Márcia V.; Silva, L. D. da; Santos, J. B. dos; Ferreira, E. A. & Santos, L. D. T. Effects of irrigation and nitrogen fertilization rates on yield, agronomic efficiency and morphophysiology in *Tithonia diversifolia*. *Agric. Water Manag.* 248:106782, 2021. DOI: https://doi.org/10.1016/j.agwat.2021.106782.

Siddaram, M. D.; Santhosh, G. P.; Shubha, S.; Gundappagol, R. C. & Umesh, M. R. Field evaluation of consortium of *Azospirillum*, PSB and AM fungus on yield parameters of direct seeded rice. *Environ. Ecology*. 35 (2C):1364-1367. https://drive.google.com/file/d/1dFhKz4-gihuVNRAd-Lj-jH_iFy5blxaI/view, 2017.

Sieverding, E.; Silva, G. A. da; Berndt, R. & Oehl, F. *Rhizoglomus*, a new genus of the Glomeraceae. *Mycotaxon*. 129 (2):373-386, 2014. DOI: https://doi.org/10.5248/129.373.

Simó-González, J.; Rivera-Espinosa, R.; Ruiz-Martínez, L. & Martín-Alonso, Gloria. La integración de inoculantes micorrizicos, abonos verdes y abonamiento orgánico-mineral en plantaciones de bananos en suelos pardos. *Trop. Subtrop. Agroecosystem*. 23 (8). https://www.revista.ccba.uady.mx/ojs/index.php/TSA/article/view/2882/1367, 2020.

Trouvelot, A.; Kough, J. L. & Gianinazzi-Pearson, V. Mesure du taux de mycorhization VA d'un système radiculaire. Recherche de méthode d'estimation ayant une signification fonctionnelle. *Physiological and genetical aspects of mycorrhizae. Proceedings of the 1st Symposium européen sur les mycorhizes.* París: INRA. p. 217-221, 1986.

Yu, Y.; Zhao, L.; Cui, M.; Xiao, Y.; Wang, C.; Miao, Y. *et al.* Exploring slope spatial heterogeneity by nitrogen transfer and arbuscular mycorrhizal community. *J. Soils Sediments*. 20:3569-3579, 2020. DOI: https://doi.org/10.1007/s11368-020-02690-4.