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COINOCULATION OF *Canavalia ensiformis* WITH *Rhizobium* AND ARBUSCULAR MYCORRHIZAL FUNGUS IN TWO SOILS FROM CUBA

Coinoculación de *Canavalia ensiformis* (L.) D.C. con *Rhizobium* y Hongos micorrízicos arbusculares en dos tipos de suelos de Cuba

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ABSTRACT. To evaluate the response of jack bean (*Canavalia ensiformis* (L.) D.C.) to the coinoculation with strains of *Rhizobium* and arbuscular mycorrhizical fungus (AMF), it was carried out an experiment under microplots conditions that contained Nitisol soil coming from the Department of Agricultural Services of the INCA, in San José de las Lajas, Mayabeque, and another soil from the Station of Grasses and Forages of Cascajal, Villa Clara (Ferruginous Nodular Gley). Four strains of *Rhizobium* were studied (Can 2, Can 3, Can 4 and Can 5) and two strains of AMF (*Glomus cubense* (INCAM 4) and *Rhizophagus intraradices*) (INCAM 11) for the Nitisol soil and *Glomus cubense* and *Funneliformis mosseae* (INCAM 2) for the Nodular Gley soil more the corresponding controls without inoculation, for a total of 15 treatments for each soil type, which were distributed in a totally randomized design with factorial arrangement (5 x 3) and three repetitions. The mycorrrhizic symbiosis indicators and the yield of dry mass were evaluated. The results showed that the jack bean responded to the coinoculation *Rhizobium*-AMF in both types of soil. The best behavior in the strains of *Rhizobium* was obtained with can 3 for the Nitisol soil and Can 3, Can 4 and Can 5 for Nodular Gley soil, and the best strain of AMF were, in that order, *G. cubense* and *F. mosseae* for one and another soil, respectively.

Key words: green manures, jack bean, seeds inoculation, nitrogen fixing bacteria, mycorrhizae

RESUMEN. Para evaluar la respuesta de la canavalia (*Canavalia ensiformis* (L.) D.C.) a la coinoculación con cepas de *Rhizobium* y de hongos micorrízicos arbusculares (HMA), se condujo un experimento en condiciones de microparcelas que contenían suelo Ferralítico Rojo Lixiviado procedente del Departamento de Servicios Agrícolas del INCA, en San José de las Lajas, Mayabeque, y suelo Gley Nodular Ferruginoso procedente de la Estación de Pastos y Forrajes de Cascajal, Villa Clara. Se estudiaron cuatro cepas de *Rhizobium* (Can 2, Can 3, Can 4 y Can 5) y tres cepas de HMA: *Glomus cubense* (INCAM 4) y *Rhizophagus intraradices* (INCAM 11) para el suelo Ferralítico Rojo y *Glomus cubense* y *Funneliformis mosseae* (INCAM 2) para el suelo Gley Nodular más los correspondientes testigos sin inoculación, para un total de 15 tratamientos por tipo de suelo, los cuales se distribuyeron en un diseño completamente aleatorizado con arreglo factorial (5 x 3) y tres repeticiones. Se evaluaron los indicadores del funcionamiento de la simbiosis micorrízica y el rendimiento de masa seca. Los resultados mostraron que la canavalia respondió positivamente a la coinoculación *Rhizobium*-HMA en ambos tipos de suelos. El mejor comportamiento de las cepas de *Rhizobium* se obtuvo con Can 3 para el suelo Ferralítico Rojo y Can 3, Can 4 y Can 5 para suelo Gley Nodular Ferruginoso, y las mejores cepas de HMA fueron, en ese orden, *G. cubense* y *F. mosseae* para uno y otro suelo, respectivamente.

Palabras clave: abonos verdes, canavalia, inoculación de semillas, bacteria fijadora del nitrógeno, mycorrhizae

INTRODUCTION

Sustainable way of incorporating nitrogen in agricultural systems is the insertion into the crop rotation, plants in symbiosis with microorganisms

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capable of FBN among these types of plants, legumes are used as green manure having several advantages because in addition to the substantial contribution of nitrogen given by them, they are able to recycle other nutrients and improve some physical and biological soil properties (1).

In Cuban conditions, one of the types of green manure that has had good results is *Canavalia ensiformis* (L.) D.C. capable of providing more than 150 kg ha^{-1} of N until 5 t ha^{-1} of dry mass, besides increasing sustainable yields of crops such as: corn, potato, pumpkin, taro, among others (2).

Arbuscular mycorrhizal fungi (AMF) form a symbiotic association among certain soil, fungi and higher plant roots. In them, both symbionts are mutually benefited. Micorrhizae receive carbonated sources from the plant while through fungal structures, the soil exploration capacity, the nutrient absorption growth and development in plants are increased (3).

In Cuba, several studies have been developed, they allow to affirm that the soil type defines which are efficient AMF species for a given edaphoclimatic condition (high specificity AMF soil-strain), although the effectiveness achieved by inoculation depends on the handling given to plant and soil. So it is reported that the strain *Rhizophagus intraradices* (INCAM 11) is the most efficient in high fertility clay soils, *Funneliformis mosseae* (INCAM 2) favors the colonization in low fertility acid soils and *Glomus cubense* (INCAM 4) specie is more effective in soils of medium to high fertility (4).

At *Rhizobium*-AMF-legumes coinoculation, it is reported that, symbiotic relationships provide greater exchange between symbionts and superior effects in plants than relations based on non-symbiotic associations. In this case the *Rhizobium*-AMF-legumes symbiosis provides N_2 and mycorrhizae increase the absorption of other elements including P, that is very important to ensure adequate FBN and plant growth (4).The *Rhizobium*-AMF double inoculation produces higher growth, number, nodule dry weight and higher contents of P and N in the plant.

Based on this, over recent years, Cuba has been developing works towards the isolation of Rhizobium strains in the canavalia rhizosphere and assessing the impact of its coinoculation with AMF effective strains in different soil types, to achieve further growth of the species and enhance their use as green manure or cover crop.

Taking into account the above this work was done with the aim of evaluating the canavalia response to inoculation of four Rhizobium strains and three AMF strains, into two soil types in Cuba.

MATERIALS AND METHODS

An experiment was carried out in conditions of microplots, containing Ferralitic Red Leachate soil (5) from the INCA Agricultural Services Department, in San José de las Lajas, Mayabeque Province and Ferruginous Nodular Gley soil (5) from the Pastures and Forages station in Cascajal, Villa Clara

The microplots are located in areas of the Biofertilizers Department and Plant Nutrition, in the National Institute of Agricultural Sciences (INCA), with a dimension of 0,63 wide meters by 2,55 long meters and 0,70 height meters, They were sown during the period from July to October, 2012, which coincides with the optimum period for planting canavalia as green manure in the region (2).

The soil was taken from the surface horizon (0-20 cm) and it was moved toward the microplots. Initial characteristics of the soil used in the experiment are shown in table. Chemical determinations were performed: pH H_2O potentiometer, organic matter by Walkley Black, P Oniani, cations NH_4Ac pH 7, Gerdemann and Nicholson AMF spore number, 1963, modified by Herrera /*et al./,* 1995 (6).

It is used as green manure *Canavalia ensiformis* (L.) DC. A completely randomized experimental design was used and three replications in a bifactorial arrangement (5 x 3). Five levels of Rhizobium inoculation factor (four strains plus a treatment without inoculation) and three levels of mycorrhizal inoculation factor (two strains and a control without inoculation) were studied for a total of fifteen treatments for each soil.

Some initial characteristics of cultivable soil horizon used in the experiment

	pH	OM (%)	P (mg kg^{-1})	Ca	Mg	K	Na	Number of AMF spores in 50 g of soil
					(cmol kg^{-1})			
Red Ferralitic	7,31	3,93	392,13	23,94	14,83	ND	ND	115
Ferruginous Nodular Gley	4,7	2,45	1,4	4,2	1,8	0,10	0,08	69

ND. Non determined OM (organic matter)

As mycorrhizal inoculum was used the commercial product EcoMic® using three species *Glomus cubense*, strain INCAM 4 (7), *Funnelifomis mosseae*, strain INCAM 2 and *Rhizophagus intraradices*, strain INCAM 11 (8): minimum guaranteed quality, 20 spores g^{-1} of inoculant, non-toxic and free of pathogens product, produced by the National Institute of Agricultural Sciences (INCA). The product contained each strain separately.

Four strains of *Rhizobium* (Can 2 Can 3 Can 4 and Can 5) from the collection of *Rhizobium* strains of the Physiology Laboratory in the National Institute of Agricultural Sciences (10) were used.

Coinoculation of these fertilizers was applied at the time of planting, by the coating seeds method (9) using the corresponding dose, 10 % of the total weight of the Canavalia inoculated seeds with each of the strains AMF and 100 g of each strain of *Rhizobium*, contained in solid support of sterile peat.

The seeds were wetted with water first and then each *Rhizobium* strain was applied, according to the treatments to be studied, using the manual coating of seeds with inoculant, later inoculation with arbuscular mycorrhizal fungi was proceeded, they began to dry in shade for 15 minutes and afterwards planting was done.

A planting of 0,40 x 0,30 m, with two furrows in each microplot, and a seed per nest, for 12 plants per furrow and a total of 24 plants per microplot was used. Cultural cares were based on recommendations from other studies (11). For taking samples, 12 plants were selected within each microplot, 70 days after germination. The complete aerial part of plants, roots and rhizosphere soil was sampled.

The spore count was performed on samples of 50 g of rhizosphere soil, judging by the extraction method described by Gerdeman and Nicholson in 1963, as amended by Herrera et al. 1995 (6), based on the sieving decanted and wet fungal propagules.

To determine mycorrhizal colonization, collected plant roots to a 15 cm depth were taken; they were washed with tap water and dried in air. Finer rootlets were taken and minced. For determinations, 200 mg of rootlets approximately were weighed, and they were dried at 70 °C, to be dyed according to the described methodology by Phillips and Hayman in 1970. The evaluation was carried out by intercept methods, developed by Giovanetti and Mosse (6).

To determine the leaf dry biomass (t ha^{-1}), the aerial organs of plants were picked (leaves and stems). The total of each organ fresh mass was weighed, separately in a Sartorius balance hence, a fresh mass fraction of 100 g was taken and put to dry in an oven at 70 °C to reach constant mass values, then dry mass was estimated and extrapolated at ha^{-1}.

For the experiment data analysis, the corresponding mathematical model to experimental design was used, with which we worked, checking the data normality and variance homogeneity. Comparison test of Duncan's multiple range for p≤0, 05 was used to determine differences between treatments. For analysis program, Statgraphics plus 5.1 for Windows was used.

RESULTS AND DISCUSSION

The effect results of the studied variables on the canavalia dry mass are appreciated in Figure 1. The analyzed factors, Rhizobium co-inoculation and AMF presented interaction among them. The dry mass highest values were observed in the *Glomus cubense* combination (INCAM 4) and Rhizobium (Can 2) in plants grown in Red Ferralitic soil. However, in the case of Ferruginous Nodular Gley soil, the best performance was obtained with the co-inoculation of Funneliformis mosseae (INCAM 2) and Can 3 Can 4 and Can 5 strains.

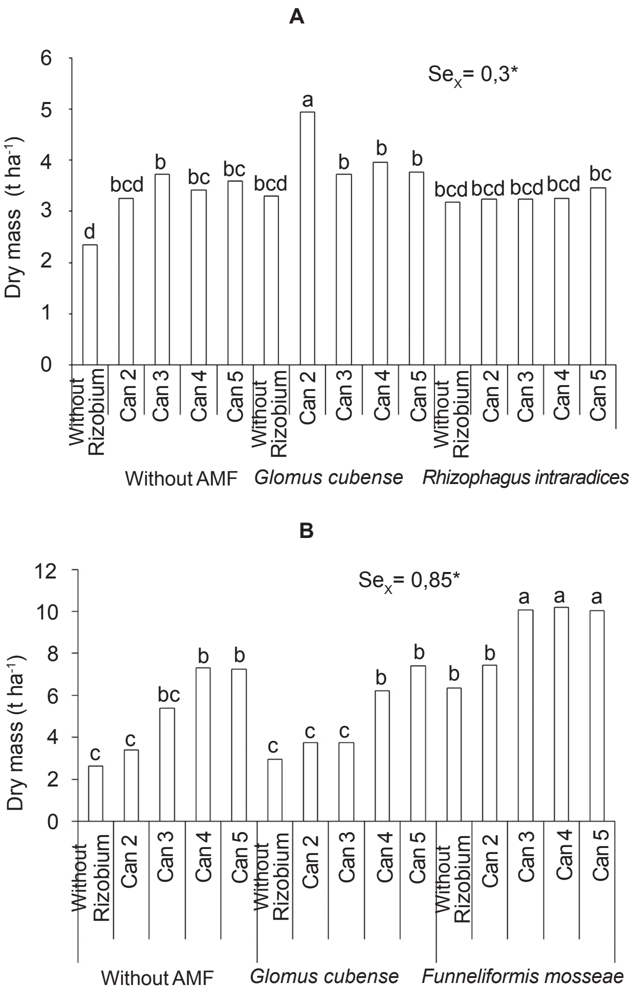
It should be noted that for both soils, canavalia had a dry mass that ranged from 2.5 to 10 t ha^{-1}. This behavior is typical of a legume that is usually used as a green manure and planted in optimum time, in this case, under high temperature and humidity conditions (summer), that makes it having lush growth (2).

Regarding the AMF strains performance, results that report high specificity AMF strain per soil type (4) are ratified. Thus, *G. cubense* species (INCAM 4) is the most effective one in Ferralitic Red soil conditions, even in environments of high tech agriculture and soil fertility (11). For Ferruginous Nodular Gley soil, marked acidity, the strain reported as most effective was precisely *F. mosseae* (INCAM 2) (12).

The effectiveness of an AMF strain is when it is able to establish a symbiosis with the host plant, having a positive influence on the growth and development of it and at the same time, multiplying its propagules in the environment where it is (13).

With regard to *Rhizobium* strains, a legume inoculation with effective strains causes an increase in the biomass of plants, although many reports indicate that canavalia is a promiscuous genre that can establish effective symbiosis with various *Rhizobium* strains (10).

The results of this study indicate that apparently, there is a relative efficiency of *Rhizobium* strains per soil type, because the strain inoculated to greater accumulation of biomass resulted in canavalia grown on Ferralitic Red soil (CAN 2) was not the same as for Ferruginous Nodular Gley soil conditions (Can 3, Can 4 and Can 5).



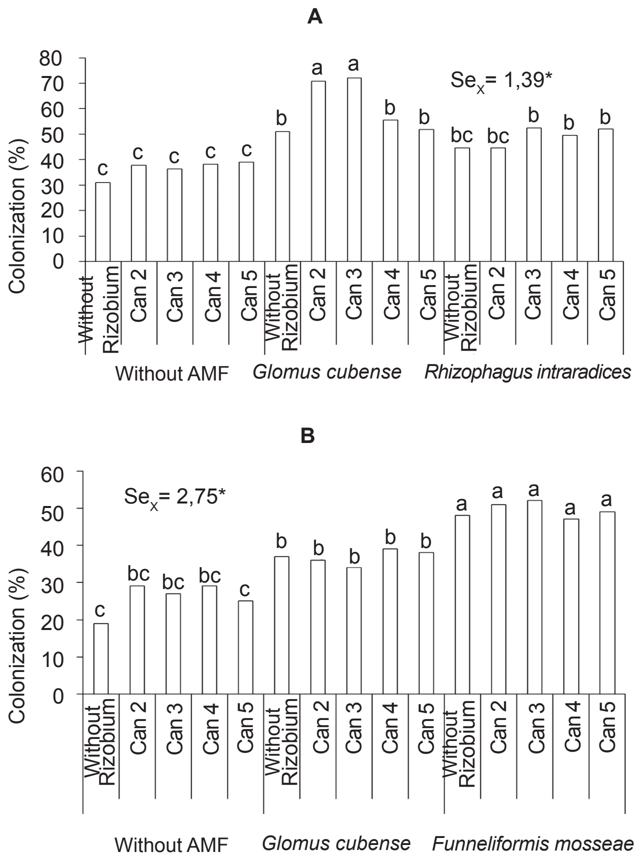
**Mean according to Duncan test (p<0, 05), with different letters differ among them. A: Ferralitic Red soil. B: Ferruginous Nodular Gley soil

Figure 1. Coinoculation effects with Rhizobium and AMF on Canavalia dry mass

In this regard, in conditions of Ocric Brown soils without carbonates in Tercer Frente, Santiago de Cuba, the Can 3 strain was the best performer, promoting significant increases in height, dry mass and canavalia nodulation and plants inoculated with the Can 5 strain had a similar behavior to uninoculated control (14).

Moreover, canavalia inoculation with Can 2, Can 3 and Can 5 strains causes a marked increase in nodulation and growth of plants, grown in Ferralitic Red soil (15). Although this study was not possible to assess variables related to nodulation and its effectiveness, these results suggest the need for further research regarding the effectiveness of Rhizobium strains being inoculated green manure species in different soil types.

In Figure 2 the interaction effect among the factors under study on percentage of canavalia mycorrhizal colonization is shown. The highest values were found in the case of Ferralitic Red soil, with treatments that combined *G cubense* inoculation with *Rhizobium* strains, Can 2 and Can 3.



*Mean According to Duncan test (p<0, 05), with different letters differ among them. A: Ferralitic Red soil. B: Ferruginous Nodular Gley soil

Figure 2. Coinoculation effect with Rhizobium and AMF on Canavalia mycorrhizal colonization percentages

For Ferruginous Nodular Gley soil, mycorrhizal colonization highest values were found in all treatments with inoculation of *F. mosseae* (INCAM 2), regardless of the *Rhizobium* inoculated strain, confirming that under high acidity conditions of these soils, this AMF strain is able to establish a symbiosis with any mycotrophic plant species (12).

However, a marked tendency to superior colonization is observed in those inoculated treatments with AMF strains with respect to those without mycorrhizal inoculation. In all cases colonization rates had values over 20 % and the most important treatments, even reaching values over 50 %.

This result indicates that despite some limiting conditions to symbiosis development, such as the high acidity of Cascajal soil, mycorrhizal symbiosis was presented.

In this regard, it has been shown that canavalia is a legume species that can reach high percentages of mycorrhizal colonization and in soil Ferralitic Red conditions the strain that stands out, not only in this variable but also stimulate further aerial growth, was Glomus *hoi like* species (16), reclassified as *G. cubense* (INCAM 4) (7).

In correspondence with these results, you can see the visual density percentage in Figure 3A, the factors under study presented interaction among them and the higher values of this variable were again found in the same more effective combinations for soil types, found by analyzing the mycorrhizal colonization percentage.

The visual density is a variable that describes the degree of intensity with which the fungus colonizes the root. In this case, the result found that mycorrhizal colonization was effective in those treatments inoculated with the most efficient strains for the soil type under study is ratified.

It should be noted that in all evaluated cases, has highlighted the Rhizobium-AMF coinoculation effectiveness, finding the highest values of evaluated variables in the presence of coinoculation, when they are compared with treatments that only inoculated a microorganism.

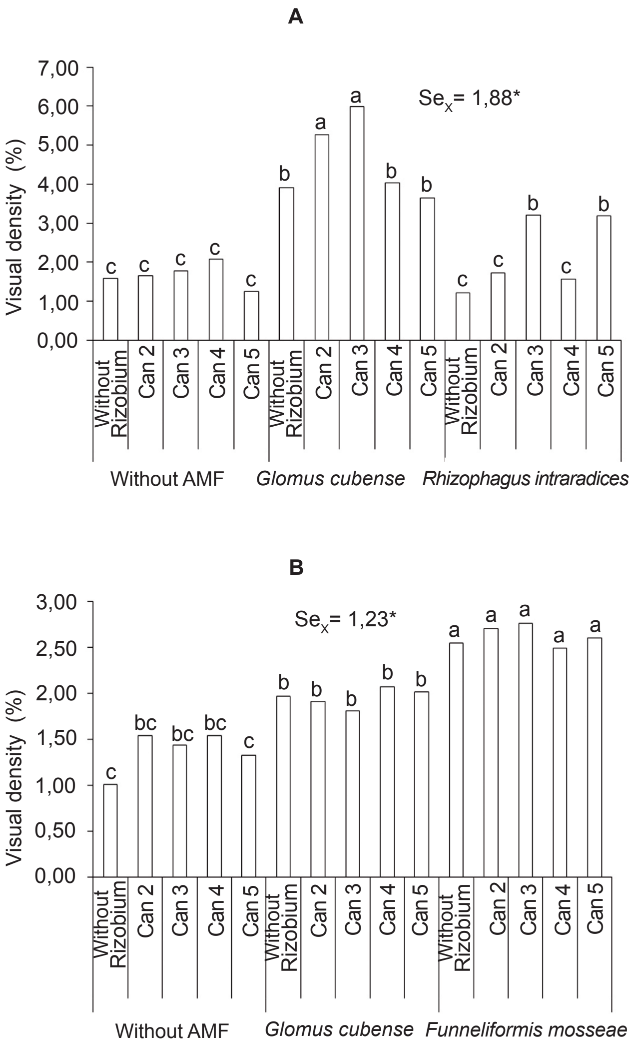
It arises about the symbiotic relationships should provide greater exchange between symbionts and higher effects to plants, that relations based on non-symbiotic associations. In this case *Rhizobium*- legume symbiosis provides N_2 and mycorrhizae increase the absorption of other elements, including P (4).

Moreover, in mycorrhizal legumes it may be an indirect effect and it is probably a response to the bacterial symbiont increased force and demand of N by mycorrhizal plants (17).

In Figure 4, the interaction effect of the studied factors on the proliferation of AMF spores present in the canavalia rhizosphere is observed. The largest numbers of spores were found in those plants that showed the highest growth in aerial biomass and fungal high performance indicators. It is to emphasize the values found, well above the initial contents of the number of AMF spores (Table).

Canavalia is a plant species that among other advantages, to be used as green manure, has the peculiarity of multiplying AMF propagules in the soil, whether native or inoculated and propitiating mycorrhizal colonization of the subsequent crop, all of which gives the plant an added value as green manure in farming systems (11).

On the other hand this result is an indicator of the Rhizobium-AMF-legume tripartite interaction benefits in which all parts are enhanced and this way can express their maximum biological and agronomic potential, all of which may affect more impact of its introduction in agricultural systems.

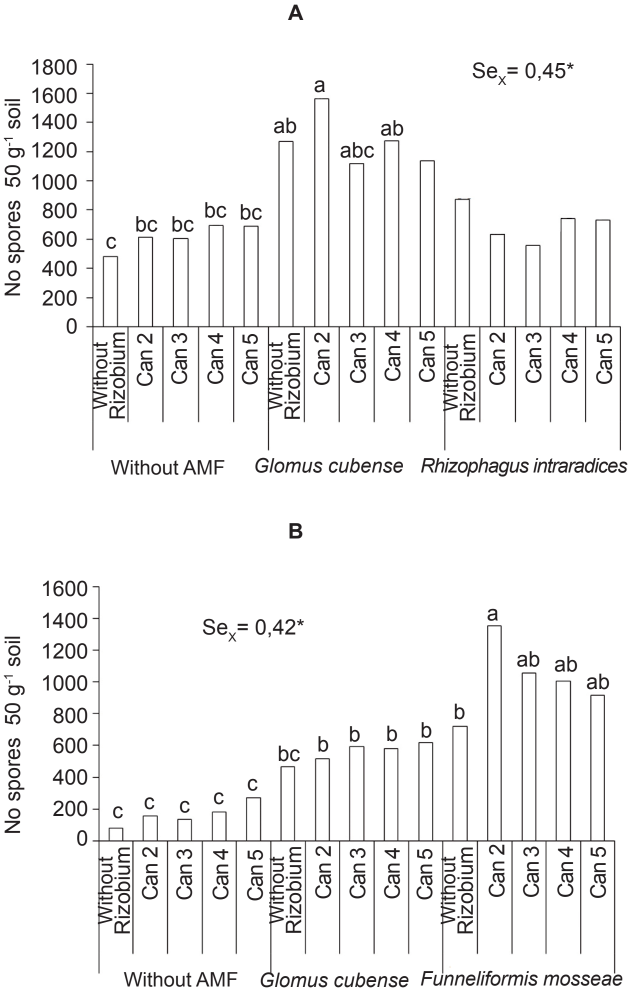


*Mean According to Duncan test (p<0, 05), with different letters differ among them
A: Ferralitic Red soil. B: Ferruginous Nodular Gley soil

Figure 3. Coinoculation effect with *Rhizobium* and AMF on the percentage of Canavalia visual density

Thus, the benefits of bacteria-AMF- legume tripartite symbiosis related to nitrogen fixing bacteria nodulation process and the establishment of arbuscular mycorrhizae can occur simultaneously and synergistically. Fungi mobilize P and other nutrients from the soil, while bacteria provide N, not only to plants but also to AMF (18).

In this manner, the AMF and nitrogen fixing bacteria may act synergistically stimulating the legumes growth by means of a nutrient acquisition (19). Furthermore, root colonization by the AMF can affect bacterial communities associated to the rhizosphere directly or indirectly.



* Mean According to Duncan test (p<0, 05), with different letters differ among them

A: Ferralitic Red soil. B: Ferruginous Nodular Gley soil

Figure 4. Coinoculation effect with *Rhizobium* and AMF on number of AMF spores present in canavalia rhizosphere

Direct interactions include energy supply by carbon-rich compounds that are transported from the host plant to the mycorrhizosphere through fungal hyphae (20). Indirect interactions refer to mycorrhiza effects on the host plant growth, the exudation of growth promoting substances and improving soil structure (21). All these factors increase the nitrogen fixing bacteria activity (22).

Integrally, AMF strains *G. cubense* (INCAM 4) in the case of Ferralitic Red soil and *F. mosseae* (INCAM 2) Ferruginous Nodular Gley soil, were the most effective, which ratified the effect of high specificity AMF-soil strain reported in previous works. On the other hand, it highlights the importance of inoculation with Rhizobium of *Canavalia ensiformis* legume, increasing the growth of it and at the same time facilitate the symbiosis with mycorrhizal fungi, which in turn will provide the necessary nutrients to plant and the biological fixation process.

The response of plants to coinoculation with various types of microorganisms can be from synergistic to antagonistic depending on fungi and bacteria strains that are used also the inoculated plant species (23). Canavalia ensiformis coinoculation with two types of beneficial soil microorganisms, in this case, a fungus and a bacterium, enhanced growth and development of the plant while, microorganisms were not antagonistic to each other, ratifying their mutual effect, which can be used effectively with a greater introduction of these practices within agricultural systems.

CONCLUSIONS

- The canavalia is a green manure that responds positively to Rhizobium-AMF coinoculation in Ferralitic Reds and Ferruginous Nodular Gley soils.
- The better performance of *Rhizobium* strains was obtained with Can 3 for Ferralitic Red soil and Can 3 Can 4 and Can 5 in the case of Ferruginous Nodular Gley one.
- The best strains were AMF *Glomus cubense* for Ferralitic Red soil and *Funneliformis mosseae* Ferruginous Nodular Gley one.
- The Rhizobium-AMF coinoculation stimulates the behavior of the indicator variables of the mycorrhizal symbiosis operation.

BIBLIOGRAPHY

1. Martín, G. M. y Rivera, R. Mineralización del nitrógeno incorporado con los abonos verdes y su participación en la nutrición de cultivos de importancia económica. *Cultivos Tropicales*, 2004, vol. 25, no. 3, pp. 89-96. ISSN: 1819-4087.
2. García, M.; Treto, E. y Álvarez, M. Época de siembra más adecuada para especies promisorias de abonos verdes en las condiciones de Cuba. *Cultivos Tropicales,* 2002, vol. 23, no. 1, pp. 5-14. ISSN: 1819-4087.
3. Veresoglou, S. D.; Chen, B. y Rillig, M. C. Arbuscular mycorrhiza and soil nitrogen cycling. Review. *Soil Biology & Biochemistry*, 2012, vol. 46, pp. 53-62. ISSN: 0038-0717.

4. Rivera, R. y Fernández, K. Bases científico-técnicas para el manejo de los sistemas agrícolas micorrizados eficientemente. En: Rivera, R. y Fernández, K. Eds. Manejo efectivo de la simbiosis micorrízica, una vía hacia la agricultura sostenible. Estudio de caso: el Caribe. INCA. La Habana. 2003. 166 pp. ISBN: 959-7023-24-5.
5. Hernández, A.; Pérez, J. M.; Bosch, D., *et al*. Nueva Versión de clasificación genética de los suelos de Cuba. La Habana: AGRINFOR, 1999. 64 pp.
6. Mujica, Y.; Medina, N.; de la N Pons, B. Efectividad de la inoculación líquida de HMA en el cultivo del tomate (*Solanum lycopersicum* L.) en suelo Ferralítico. Editorial Académica Española. 2011. 75 pp. ISBN: 978-3-8443-3983-3.
7. Rodríguez, Y.; Dalpé, Y.; Séguin, S.; Fernández, K.; Fernández, F. y Rivera, R. A. *Glomus cubense* sp. nov., an arbuscular mycorrhizal fungus from Cuba. *Mycotaxon*, 2011, vol. 118, pp. 337-347. ISSN: 2154-8889.
8. Schußler, A. y Walker, C. 7. Evolution of the 'Plant-Symbiotic' Fungal Phylum, Glomeromycota. En: Evolution of Fungi and Fungal-Like Organisms, The Mycota XIV. Poggeler, S. and Wostemeyer, J. (Eds.). Springer. Verlag Berlin Heidelberg. 2011, pp. 163-185. ISBN: 0186-3231.
9. Fernández, F.; Gómez, R.; Vanegas, L. F.; Martinez, M. A.; de la Noval, B. M. y Rivera, R. Producto inoculante micorrizógeno. Oficina Nacional de Propiedad Industrial. 2000. Cuba, Patente No. 22641.
10. Hernández, I.; Nápoles, M. C. y Hernández, A. Biofertilizante Azofert®. Nuevas aristas y patrones de calidad. En: Congreso Científico del INCA (18: 2012, nov 6-9, Mayabeque) Memorias. CD-ROM. Instituto Nacional de Ciencias Agrícolas. ISBN: 978-959-7023-62-3.
11. Martín, G. M.; Rivera, R.; Pérez, A. y Arias, L. Respuesta de la *Canavalia ensiformis* a la inoculación micorrízica con *Glomus cubense* (cepa INCAM-4), su efecto de permanencia en el cultivo del maíz. *Cultivos Tropicales*, 2012, vol. 33, no. 2, pp. 20-28. ISSN: 1819-4087.
12. Ramírez, J. F.; Salazar, X.; González, P. J. y Rivera, R. Validación del uso de hongos micorrizicos arbusculares en la rehabilitación de pastizales. En: Congreso Científico del INCA (18: 2012, nov 6-9, Mayabeque) Memorias. CD-ROM. Instituto Nacional de Ciencias Agrícolas. ISBN: 978-959-7023-62-3.
13. Janos, D. P. Plant responsiveness to mycorrhizas differs from dependence upon mycorrhizas. *Mycorrhiza*, 2008, vol. 17, pp. 75-91. ISSN: 0940-6360.
14. Bustamante, C.; Pérez, A.; Viñals, R.; Rivera, R.; Pérez, G.; Rodríguez, M. Efecto de la inoculación con cepas de *Rhizobium* sobre indicadores de crecimiento y producción de biomasa por *Canavalia ensiformis* intercalada con cafeto en suelo Pardo de Cuba. En: Congreso Científico del INCA (18: 2012, nov 6-9, Mayabeque) Memorias. CD-ROM. 2012. Instituto Nacional de Ciencias Agrícolas. ISBN: 978-959-7023-62-3.
15. Pérez, G.; Nápoles, M. C.; Martín, G. M.; Morales, B. y Reyes, R. Aislamiento, caracterización y selección de aislados de rizobios promisorios para la inoculación de *Canavalia ensiformis*. En: Congreso Científico del INCA (16: 2008, nov 24-28, La Habana). Memorias. CD-ROM. 2008. Instituto Nacional de Ciencias Agrícolas. ISBN: 978-959-16-0953-3.
16. Martín, G. M., Arias, L. y Rivera, R. Selección de las cepas de HMA más efectivas para la *Canavalia ensiformis* cultivada en suelo Ferralítico Rojo. *Cultivos Tropicales*, 2010, vol. 31, no. 1, pp. 27-31. ISSN: 1819-4087.
17. Erman, M.; Demir, S.; Ocak, E.; Tüfenkc, S.; Oguza, F. y Akköprüb, A. Effects of *Rhizobium*, arbuscular mycorrhiza and whey applications on some properties in chickpea (*Cicer arietinum* L.) under irrigated and rainfed conditions. 1-Yield, yield components, nodulation and AMF colonization. *Field Crops Research*, 2011, vol. 122, pp. 14-24. ISSN: 0378-4290.
18. Rabie, G. H.; Aboul-Nasr, M. B.; Al-Humiany, A. Increased salinity tolerance of cowpea plants by dual inoculation of an arbuscular mycorrhizal fungus *Glomus clarum* and a nitrogen-fixer *Azospirillum brasilense*. Mycobiology, 2005, vol. 33, no. 1, pp. 51-60. ISSN: 0568-2517.
19. Sarabia, M.; Madrigal, R.; Martínez, M. y Carreón, Y. Plantas, hongos micorrízicos y bacterias: su compleja red de interacciones. *Biológicas*, 2010, vol. 12, no. 1, pp. 65-71.
20. Bonfante, P.; Anca, I. Plants, mycorrhizal fungi and bacteria: a network of interactions. *Annual Rev. Microbiol.,* 2009, vol. 63, pp. 363-383. ISSN: 1545-3251.
21. Antoun, H.y Prévost, D. Ecology of plant growth promoting rhizobacteria. En: PGPR: Biocontrol and Biofertilization. Ed. Siddiqui, Z. A. The Netherlands. 2005, pp. 1-38. ISBN: 978-1-63321-051-6.

22. González, P. J.; Pérez, G.; Medina, N.; Crespo, G.; Ramírez, J. F. y Arzola, J. Coinoculación de cepas de rizobios y una cepa de hongo micorrízico arbuscular (*Glomus cubense*) y su efecto en kudzú (*Pueraria phaseoloides*). Nota técnica. *Revista Cubana de Ciencia Agrícola,* 2012, vol. 46, no. 3, pp. 331-334. ISSN: 2079-3472.

23. Weber, J.; Ducousso, M.; Tham, F.Y.; Nourissier-Mountou, S.; Galiana, A.; Prin, Y. y Lee, S. K. Co-inoculation of *Acacia mangium* with *Glomus intraradices* and *Bradyrhizobium* sp. in aeroponic culture. *Biol Fertil Soils*., 2005, vol. 41, no. 4, pp. 233-239. DOI 10.1007/s00374-005-0833-z.

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