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INOCULATION OF ARBUSCULAR MYCORRHIZAL FUNGI AND PLANT GROWTH PROMOTING BACTERIA IN PEANUT CROP (*Arachis hypogaea* L.)

Inoculación de hongos micorrízicos arbusculares y bacterias promotoras del crecimiento vegetal en el cultivo de maní (*Arachis hypogaea* L.)

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ABSTRACT. Peanut (*Arachis hypogaea* L. cv Spanish-Valencia) is a crop that has a great commercial relevance and a high nutritional value. In order to evaluate arbuscular mycorrhizal fungi (*Glomus cubense*) effect and plant growth promoting rhizobacterium in peanut crop, this research in La Esperanza farm was developed. *Glomus cubense* strain was reproduced by arbuscular mycorrhizae laboratory and AZOFERT® was obtained from Physiology and Biochemistry Vegetable Department. Four treatments were used: absolute control, *Glomus cubense*, AZOFERT® and *Glomus cubense* + AZOFERT®. Two evaluation were carried out one to 45 days after seeding and the other one in the harvest. Fungal, growth and nodulation indicators and yield were determined. Data were analized by STATGRAPHICS statistic program for Windows. Results showed positive effect of *Glomus cubense* and AZOFERT® inoculations. *Glomus cubense* + AZOFERT® coinoculation obtained 1658 kg ha^{-1} yield. Application of these products can be an alternative to peanut production in current conditions.

Key words: stimulants, grains, nutrition, yield

RESUMEN. El maní (*Arachis hypogaea* L. cv Spanish-Valencia) es un cultivo que posee una gran relevancia comercial y un alto valor nutritivo. Con el objetivo de evaluar la influencia (*Glomus cubense*) de la inoculación de hongos micorrízicos arbusculares y de rizobacterias promotoras del crecimiento vegetal en el mismo, se desarrolló esta investigación en la Finca "La Esperanza", San José de las Lajas. La cepa utilizada fue HMA *Glomus cubense* reproducida en el laboratorio de micorrizas arbusculares y el AZOFERT® obtenido en el departamento de Fisiología y Bioquímica Vegetal del (INCA). Se utilizaron cuatro tratamientos: testigo absoluto, *Glomus cubense*, AZOFERT® y *Glomus cubense* + AZOFERT®. Se realizaron dos evaluaciones: una a los 45 días después de la siembra y en la cosecha. Se determinaron indicadores fúngicos, de nodulación, crecimiento de las plantas y rendimiento del cultivo. Los datos se procesaron a través del programa estadístico STATGRAPHICS para Windows. Los resultados mostraron el efecto positivo de las inoculaciones con *Glomus cubense* y AZOFERT®. Esta alcanzó un rendimiento de 1 658 kg ha^{-1}. La aplicación de estos inoculantes constituye una alternativa para la producción de maní en las condiciones actuales.

Palabras clave: estimulantes, granos, nutrición, rendimiento

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is a crop that has great commercial relevance and in the last decade has increased efforts to increase its production volumes. Among its main uses stands out its role in human food, since its seeds are used to produce peanut butter and oil; in animal feed is considered a high quality protein source. Among the countries with the highest production volumes is China with 39,9 million metric tons, followed by India and the United States.

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With 71 % of the world exports, Argentina, the United States, Sudan, Senegal and Brazil stand out; while India, Vietnam and some African countries periodically enter the world market according to their demand (1).

In the case of Cuba, peanut production focuses on small producers with low inputs and not all apply fertilizers. Therefore, the increase in yield is the end result of a group of interactions involving genotype, climate, soil, crop management and adequate nutrient supply (2).

Associated with the rhizosphere, there are several microorganisms, whose ability to promote the growth of crops of interest, through the production of phytohormones, the contribution of nutrients to the soil or plants, or the prevention of fungal diseases (2, 3); can be exploited as a sustainable strategy to increase peanut productivity. Therefore, one of the most valuable elements to consider in sustainable Agriculture is the use of biofertilizers, which are a viable and important alternative for achieving an agricultural development that allows the production at low cost, without contaminating the environment and conserving the fertility and soil biodiversity (4).

Among these microbial groups, plant growth promoting bacteria (PGPR, according its acronyms in Spanish) and arbuscular mycorrhizal fungi (AMF) are shown to act as coordinates in the soil-root interface (5, 6).

The peanut, like other legumes, establishes symbiosis with bacteria of the genus *Rhizobium*, which allows the crop to fix the atmospheric nitrogen, as well as to optimize the doses of nitrogen fertilizer; In this sense, some studies demonstrate the effectiveness of this process in the cultivation of soybean (*Glycine max* L.) (7, 8).

On the other hand, arbuscular mycorrhizal fungi (AMF) play an important role in mineral nutrition (6, 9, 10) and in recent years, research has been carried out to support the positive effect of inoculation of these symbionts on avocado nurseries (11), in vegetables such as tomato under conditions of abiotic stress (12), in pastures like *Brachiaria* (13); However, in the case of peanut cultivation the results are limited. The simultaneous combination of PGPR and AMF has induced synergism, which has been reflected in increased growth, nitrogen and phosphorus content in plants and production compared to those inoculated separately (7).

In Cuba, a strategy has been developed in order to increase yields in prioritized crops, which provides related producers with a technological package with inputs necessary to guarantee food production. This mainly responds to bean cultivation and in many cases, delivery is not done in time, an issue that compromises the productive process. For the case of peanuts, this benefit is not available, but even so in the country many producers, despite the difficulties, allocate areas on their farms or their sowing, so it is necessary to search for nutritional alternatives that favor the growth and yield of the crop.

The objective of the present study was to evaluate the influence of the inoculation of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria on the cultivation of peanuts (*Arachis hypogaea* L.).

MATERIALS AND METHODS

Characterization of the experimental area

The study was developed in the areas of the "La Esperanza" farm, belonging to the CCS "Nelson Fernández", San Jose de las Lajas, Mayabeque. The type of soil used was classified as Ferralitic Red leachate (14), it was correlated with ferralitic Nitisol (eutric, rhodic) (15) and in Table I some of its chemical characteristics are described at 0-20 cm depth as well as the number of AMF resident spores (50 g of soil^{-1}).

The experiment was carried out during the years 2014 and 2015 and soil preparation was carried out in the dry season to ensure successful planting in the rainy season (May-October).

Table I. Chemical characteristics of the soil corresponding to the experimental area and number of resident AMF spores

Variables	pH	OM (%)	P (mg kg^{-1})	Ca	Mg (cmol kg^{-1})	K	Na	Sum of bases	Spores AMF/50 g
	6,9	1,07	173	10,5	3,0	0,45	0,06	14,01	15

Chemical determinations: pH to H2O, Potentiometer; Organic matter (OM), Walkley Black; Phosphorus (P), Oniani; Cations, Ca2+, Mg2+, Na1+ and K1+, Maslova method; AMF spores (16) with modifications (17)

Description of Inoculants

The fungal species *Glomus cubense* (Y. Rodr. & Dalpé) (18) and the liquid inoculant AZOFER® (Rhizobium-peanut) were used; both at the National Institute of Agricultural Sciences (INCA); one in the arbuscular mycorrhizae laboratory and the other in the Plant Physiology and Biochemistry respectively. The inoculation of the first was done by the technique of coating the seeds at the time of planting and certified inoculum was used with a purity of 20 spore g of soil^{-1} and the one of the second one from strains previously isolated from the rhizosphere of this crop. The latter had a concentration of 108 colony forming units (UFC, according its acronyms in Spanish).

Experimental Design

This experiment was set up in a randomized block design with four replications, 5x3 m^2 plots, with a separation between them of 1 m for a total area of 400 m^2 and seven rows per plot with a distance between 40 cm. The seeds were previously disinfected with a solution of 10 % sodium hypochlorite (19) and placed in the shade until uniform drying was achieved. Seeding was done manually, in the early hours of the morning and the seeds were sown to a depth of 5-10 cm.

Four treatments were used: T1(absolute control), T2 (AZOFERT®), T3 (AMF-*Glomus cubense*) and T4 (AZOFERT® + AMF-*Glomus cubense*). The application of urea alone was carried out at the time of planting in the treatments inoculated and coinoculated with the microorganisms in a dose of 300 g.

Variables analyzed

Fungal indicators: the roots were washed with abundant common water, placed in a stove at 70 °C until reaching constant weight, and then stained (20). Samples were read in a stereoscope (Carl Zeiss, Stemi 2000-C/50x) and then the frequency and intensity indicators of colonization were estimated according to the methodology described in the Procedures Manual (21).

Nodule quantification: the roots were washed to be devoid of soil, once they were cleaned, the number of nodules in the main root (NRP), secondary root (NRS) and total nodules (NT) were determined.

Plant leaf indices: for determination of aerial dry mass (ADM) (g) the samples remained in the oven at 70 ºC until obtaining constant weight.

Yield: weight of 100 grains (g), number of legumes per plant and yield (kg ha^{-1}) were determined. The Efficiency Index (EI) of the co-inoculation was determined for yield, using the following equation (22):

$$\frac{\text{Rend. tratam. coinoculad - Rend. Testigo}}{\text{Rend. testigo}} \times 100$$

Statistical analysis

The data were processed by analysis of variance, according to the classification model double the original data, considering the experimental design used, and the averages were compared by means of Duncan's score for 5 % of significance (23), after verifying that they met with the adjustment of normal distribution and homogeneity of variances. Statistical software STATGRAPHICS for Windows was used (24).

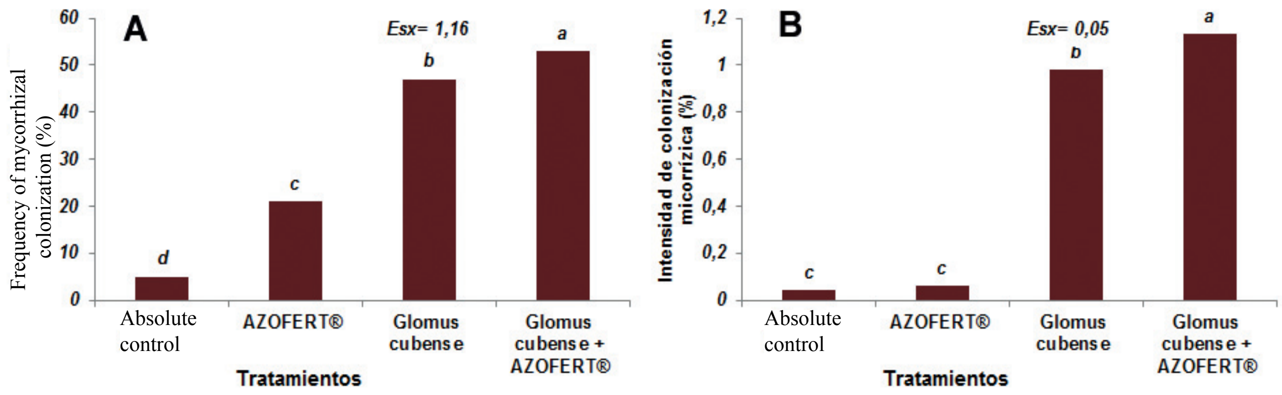
RESULTS AND DISCUSSION

Fungal Indicators

Figure 1 shows the behavior of fungal indicators evaluated in peanuts at 45 days after sowing. For the case of the frequency of mycorrhizal colonization (Figure 1A), significant differences were found between the treatments studied, being the coinoculated treatment with *Glomus cubense* + AZOFERT®, which showed the highest values (54 %). On the other hand, the treatment inoculated with *Glomus cubense* reached values of 48 %, differing significantly from the coinoculated treatment; w hile the treatment inoculated with AZOFERT® reached values of 21 %; this was significantly higher than the control treatment (5 %).

The intensity of mycorrhizal colonization is an indicator that allows to evaluate the percentage amount of fungal structures inside a mycorrhized root and its behavior at 45 DAS in the peanut crop is shown in Figure 1B. It was possible to verify the existence of significant differences between the studied treatments, being the variant coinoculated with *Glomus cubense* + AZOFERT® that reached values higher than 1,15 %. The treatment inoculated with the AMF strain differed significantly from the coinoculated treatment and reached 0,97 %. For the control treatment and inoculation with AZOFERT®, no significant differences were found and reached values of 0,12 %.

The microbial communities associated with the root system are considered to play a key role in the development of sustainable agricultural practices, because in many cases they can have synergistic effects.



A: Frequency of colonization (%) B: Intensity of colonization (%)

Figure 1. Behavior of fungal indicators in peanut cultivation at 45 DAS

The response found in fungal indicators for treatments three and four could be related to the fact that AMFs release compounds into the rhizosphere capable of attracting other microorganisms favoring the colonization of the plant (25).

On the other hand, the response found in the absolute and AZOFERT® inoculated treatments for the mycorrhizal function indicators could be related to the presence of less competitive fungal structures resident in the soil where this study was carried out. An integral analysis between both indicators (frequency and intensity of mycorrhizal colonization) shows that the co-inoculation enhanced the fungal activity of the *Glomus cubense* strain.

Another element favoring the activity of this fungus was the initial nutritional status of the soil, since in evaluating the results an average fertility could be observed (Table I) and for these conditions inoculation of this strain is recommended (26).

Studies carried out by these authors, in different crops (tarot, banana and sweet potato), demonstrated a low specificity strain of AMF-cultivation, being the high specificity strain-type soil that determines the efficiency of the fungus.

Other evidence indicates that an increase in soil fertility may, in turn, produce the opposite effect and convert this mutualist relationship into parasitic (27).

Inoculation with AZOFERT®

As for the nodulation efficiency indicators, significant differences were found between the different treatments under study (Table II). As can be seen, the treatment inoculated with AZOFERT® and coinoculated (*Glomus cubense* + AZOFERT®) reached higher values and did not differ each. On the other hand, the one that was inoculated with *Glomus cubense* obtained lower values, when compared with those obtained by the two and the four, but the same ones in turn, surpassed to the witness.

Table II. Behavior of nodulation efficiency in the treatments under study

Treatments	Main root (Rp)	Secondary root (Rs)	Total/ Nodules
Absolute control	16,41[c]	6,84[c]	23,25[c]
AZOFERT®	86,73[a]	36,94[a]	123,67[a]
AMF (*Glomus cubense*)	28,82[b]	20,21[b]	49,03[b]
AZOFERT® + AMF (*Glomus cubense*)	89,91[a]	35,09[a]	125,00[a]
EsX	7,23(*)	4,41(*)	9,11(*)

Equals with equal letters for each column did not differ significantly for Duncan (p <0.05)

In the case of total nodules, the values reached in the treatment with AZOFERT® and the coinoculated were 123,67 and 125,00 respectively, while the inoculated with AMF only obtained 49,03 and the control 23,25. While for the total nodules in the primary root the values obtained by treatments two and four were 86,73 and 89,91 respectively; on the other hand, in the three were reached of 28,82 and in the one of 16,41. On the other hand, the numbers obtained by treatment with AZOFERT® and the coinoculated in the indicator of total nodules in the secondary root were 36.94 and 35.09 respectively and for the inoculation with *Glomus cubense*, 20,21 and 6,84 for the control.

Rhizobium is a common inhabitant in agricultural soils. Often their population is insufficient to reach a beneficial relationship with the legume; therefore, when those in the soil (resident rhizobia) do not fix sufficient N amounts for legumes, it is necessary to inoculate the seed before sowing to ensure the biological fixation of the nitrogen (25). Therefore, although peanut cultivation has the ability to establish symbiosis with *Rhizobium* residing in soils, in the present study it was verified that the response found in treatments one and three for nodulation indicators could be related with the presence of this genus in the soil; However, the results demonstrated their low infectivity capacity when compared to the other variants under study.

It is valid to emphasize that an element that determines the efficiency in the interaction *Rhizobium* -legume is the dependence of the plant and the bacterium, because the plant excretes secondary metabolites towards the rhizosphere, among them flavonoids, that determine the induction of the genes Nodulation of the rhizobium, production of nodulation factors, adhesion of microbial cells to the root, induction of cell division in the plant, followed by penetration of the microsymbiont, to the formation of the symbiosis and its functioning within the Node (5). Likewise, other studies have shown that AMF, specifically its external mycelium and spores, induce changes in the composition of root exudates that favor the structure of the bacterial community of the rhizosphere (28).

Also, the response obtained in the coinoculated treatment is linked to the fact that the mutualist relationships established between mycorrhizae and plant growth promoting bacteria are given, on the one hand by the bacteria, which make the fixed atmospheric nitrogen available and, on the other hand, By mycorrhizae, which increase the absorption of other elements, among which we can mention phosphorus, a very significant element to ensure adequate nitrogen fixation and plant growth (27).

Behavior of inoculation in growth and development

The inoculation behavior on the growth and development indicators of the dry mass of the culture at 45 DAS is shown in Figure 2. As can be seen in the same, the treatment coinoculated with the AMF *Glomus cubense* strain and AZOFERT reached values greater than 60 g; that of the simple inoculated treatments did not differ significantly with each other with values of 33 g; while the absolute control differed from all study variants and reached lower values (25 g).

Aerial dry mass is one of the indicators that allows to verify the benefit obtained when using microbial inoculants, because the growth and development of the plants are stimulated, reaching significant increases. Probably the response obtained for this indicator in peanut cultivation coincides with research carried out to evaluate the influence of maize interaction, associated with pulses inoculated with *Rhizobium*, in which it was possible to increase the dry mass in the corn crop, rotated seed with inoculated legumes (29).

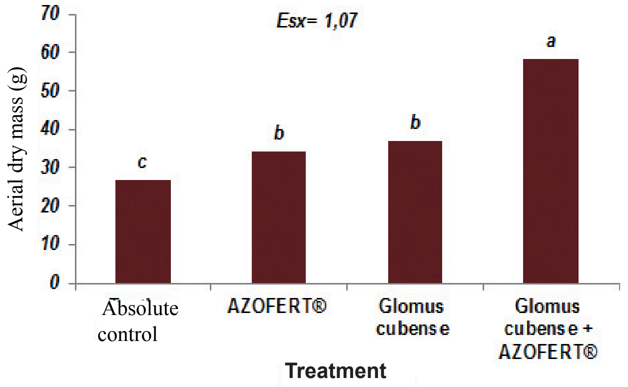


Figure 2. Influence of inoculation on aerial dry mass (g) of peanut cultivation at 45 DAS

Behavior of inoculation on yield and some components

Performance behavior and some of its components are shown in Table III. For the variable mass of 100 grains, it was possible to verify the existence of significant differences between the treatments under study, with an equal level of significance, the treatment inoculated with AZOFERT® and the coinoculated (*Glomus cubense* + AZOFERT®), with values of 1 546 and 1560 g respectively. In which the AMF strain was inoculated, a promising response could be observed for this indicator, in which values were reached that surpassed the absolute control.

Regarding yield, a differentiated behavior was found among the studied treatments, with emphasis on coinoculated treatment with values of 1 658 kg ha^{-1}. The treatment inoculated with AZOFERT® obtained yield values of 1 486 kg ha^{-1} and differed significantly from the variant inoculated with AMF (1 238 kg ha^{-1}), whereas the absolute control treatment reached values lower than 856 kg ha^{-1}. Regarding the efficiency index (EI) of the coinoculation, with respect to the control treatment, values of 93 % were found.

Another distinctive element that determines the efficiency of the microbial inoculant application and their relation with the growth, development and yield of plants are the climatic conditions. In Cuba, as in many tropical regions, farming is favored in the rainy season. This is due not only to the higher amount of precipitation, but also to the higher levels of temperature and humidity that occur during this period, which could determine the response in inoculations of microorganisms (1).

Table III. Behavior of performance and some components during the experiment

Treatments	Mass 100 grains (g)	Nu. legumes/ plants	Yield (kg ha^{-1})
Absolute control	691[c]	8,21[d]	856[d]
AZOFERT®	1 546[a]	15,1[b]	1 486[b]
AMF (*Glomus cubense*)	1 249[b]	12,44[c]	1 238[c]
AZOFERT® + AMF (*Glomus cubense*)	1 560[a]	17,36[a]	1 658[a]
EsX	0,31(*)	0,06(*)	0,09(*)

Equals with equal letters for each column did not differ significantly for Duncan (p <0.05)

This, together with the increase in the volume of soil exploration, greater translocation of water and nutrients from the soil to the plant, favors its nutritional status and, therefore, its growth and development (2).

CONCLUSIONS

- The application of *Glomus cubense* and AZOFERT® was effective in peanut cultivation when evaluating performance indicators for each symbiont, but co-inoculation significantly increased air dry mass and yield, with an efficiency index of 93 %.
- The use of arbuscular mycorrhizal fungi and plant growth promoting bacteria is an ecological alternative to increase yields and favor soil conservation and protection.

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