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Original article

# Arbuscular mycorrhizogenic fungi and organic fertilizer levels in coconut seedling breeding

## Hongos micorrizógenos arbusculares y niveles de abonado orgánico en la obtención de posturas de coco

**Blanca M. de la Noval-Pons**[1*], **Karen Alvarado-Ruffo**[2], **Albaro Blanco-Imbert**[3], **Gloria M. Martín-Alonso**[1], **Eduardo Furrazola**[4], **Ramón Capdesuñer-Rojas**[5], **Keyler Matos-Thompson**[6]

[1]Instituto Nacional de Ciencias Agrícolas (INCA), carretera San José-Tapaste, km 3½, Gaveta Postal 1, San José de las Lajas, Mayabeque, Cuba. CP 32 700

[2]Centro de Desarrollo de la Montaña, Departamento Ejecutivo de la Filial Provincial ACTAF, Luz Caballero esquina 2 Sur, Guantánamo

[3]Instituto de Suelos Guantánamo, Departamento de Investigación. Carr. 8 Este. Ciudad Deportiva, Guantánamo

[4]Instituto de Ecología y Sistemática, Carretera de Varona No. 11835 entre Oriente y Lindero, Calabazar, Boyero, La Habana, Cuba. CP 11900

[5]Empresa Agropecuaria y Coco Baracoa, La Playa, Baracoa, Guantánamo

[6]Centro de Desarrollo de la Montaña. Departamento de Tecnología Integral para la Producción Agrícola. Limonar de Monte, El Salvador, Guantánamo

**ABSTRACT:** The Arbuscular Mycorrhizal Fungi (AMF) perform an important function, when contributing of more efficient form to the survival and growth of nursery, when playing a crucial role in plant nutrition. The aim of the present study was to evaluate three AMF strains inoculated in coconut “Indio Verde-1” nursery in combination with organic mature levels in 0 soils Arenosol haplic (ARh) and haplic Fluvic Gleysol (GFLh), in Baracoa, Guantánamo. The results demonstrated to differential response to AMF species with different organic mature levels. The best results were obtain with the low levels of paid organic S:H:FC, 10:1:1 and 4:1:1. *R. irregularis* strains showed similar results to the controls in ARh soil (breeding ground to Playa Duaba), whereas in the GFLh soil (breeding ground to Cabacú) the three AFM strain to obtain similar response. With the AMF use, it is manage to replace 75 % of organic mature.

**Key words:** *Cocos nucifera*, mycorrhizae, nursery, AMF.

**RESUMEN:** Los hongos micorrízicos arbusculares (HMA) desempeñan una importante función, al contribuir de forma más eficiente a la supervivencia y crecimiento de las posturas, al jugar un papel crucial en la nutrición de las plantas. El objetivo del presente estudio fue evaluar tres cepas de HMA inoculadas en plántulas de coco “Indio Verde-1”, en combinación con niveles de abonado orgánico en dos suelos, Arenosol háplico (ARh) y Gleysol Flúvico háplico (GFLh), en Baracoa, GuantánamoLos resultados demostraron una respuesta diferente de la interacción de HMA con los diferentes niveles de abonado orgánico. Los mejores resultados se obtuvieron con los niveles más bajos de la combinación de Suelo: Humus de lombriz: Fibra de Coco (S:H:FC), 10:1:1 y 4:1:1. En el suelo ARh (vivero de Playa Duaba), la cepa *R. irregularis* mostró resultados similares a los controles, mientras que en el suelo GFLh (vivero de Cabacú), las tres cepas obtuvieron similar respuesta. Mediante el empleo de los HMA se logró sustituir el 75 % del abono orgánico.

**Palabras clave:** *Cocos nucifera*, Mycorrhizae, viveros, HMA.

*Author for correspondence: bdelanov@inca.edu.cu

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## INTRODUCTION

One of the most important terrestrial symbioses is that which occurs between 85 % of plants and fungi of the phylum Mucoromycota, including Glomeromycotina, a subphylum to which arbuscular mycorrhizal fungi belong, which are associated with 74 % of terrestrial plants (1). The coconut palm is among the group of crops that naturally establish symbiosis with this group of fungi, in which there is a great diversity of autochthonous species (2). In Cuba, Baracoa municipality is responsible for 85 % of the national coconut production, with an average yield of 2.2 t $ha^{-1}$ (3), which has been limited by Climate Change effect, as well as the presence of diseases and the palm substitution by other crops, the monoculture persistence in most plantations, the existence of aged plantations, which together with the failure in seed selection, has reduced the plantation areas (4).

AMF play an important role, contributing in a more efficient way to the survival and growth of seedlings, playing a crucial role in plant nutrition (5,6), being identified different transporters, related to the symbiosis, linked to the movement of phosphorus, nitrogen, zinc, iron, as well as sugars (7). In mycorrhizal plants there is an increase in the retention and efficient use of water (8), and at the same time they allow overcoming abiotic (9,10) and biotic (11,12) stresses by inducing defense responses in the plant (13,14). The present work was carried out with the aim of evaluating the AMF strain inoculation influence in obtaining coconut seedlings, combined with different concentrations of organic fertilizer and its interaction with native strains, in two production nurseries.

## MATERIAL AND METHODS

The experiment was developed in the nurseries of Playa Duaba, with a haplic Arenosol (ARh) soil; and Cabacú, with a Fluvic Gleysol (GFL) soil (15), both in Baracoa municipality.

As substrate was used the combination Soil: Worm humus: Coconut fiber (decomposed) (S:H:FC), of which 3 combinations of organic fertilizers and soil were studied (2:1:1, 4:1:1:1 and 10:1:1:1) (16). In each locality, the own soil was used, from which the chemical characterization of the cultivable horizon (0-0.20 m) was carried out (17) and the AMF spore content was determined (18).

The domesticated ecotype of coconut palm "Indio Verde-1" (19) was used, whose seeds were obtained from mother plants, which were prepared and sown as described (16).

Three species of AMF were selected, which came from the stock of the National Institute of Agricultural Sciences (INCA): *Glomus cubense*, Y. Rodr. and Dalpé (20) strain INCAM-4 (DAOM241198), with 92 spores $g^{-1}$ inoculum; *Rhizophagus irregularis* (Blaszk, Wubet, Renker and Buscot) Walker and Shüßler (21) strain INCAM-11 (DAOM711363), with 73 spores $g^{-1}$ inoculum and *Funneliformis mosseae*, (Nicol. and Gerd.) Walker and Schüßler (21) strain INCAM-2, with 57 spore $g^{-1}$ inoculum. Inocula were adjusted and applied on seed as referred to (4).

Additionally, three controls were used, consisting of mineral fertilization (NPK 100 %) for which the complete formula 9:13:17 was used, at a rate of 45 g per seed, fractionated at 33 % at 30 days after sowing (das) and the rest at 90 das the substrate S:H:FC 1:1:1:1 v/v/v/v and soil (16).

A randomized block design with bifactorial arrangement (3x3) and three replications was used. An experimental plot of 1.25 $m^2$ was used on a bed of plants, which consisted of five rows containing 20 seeds sown at a distance of 0.05 x 0.20 m. Each row constituted a replicate of one experimental plot. Each row constituted a replicate.

The experiment lasted 180 days, at which time 15 plants per replicate were sampled and the height (cm), diameter at stem base (cm) and N, P and K contents (g $plant^{-1}$) in the aerial part were evaluated. At 120 das, the germination velocity index (GVI) was calculated (16). The quantification of mycorrhizal variables, colonization percentage (% C) (22), and mycorrhizal colonization intensity (%) was also carried out (18). The experiment was repeated for two years.

Data were processed by Simple Rank Analysis of Variance and Duncan's Multiple Range Test at 95 % significance level. Data for the variable percentage of mycorrhizal colonization were transformed by the formula Arcseno$\sqrt{x}$. The statistical package STATGRAPHIC version 15.2 was used.

## RESULTS AND DISCUSSION

In the first year, the characterization of the cultivable horizon (0-0.20 m) of soils existing in the areas where the experiment was carried out was performed, whose results are shown in Table 1, in which it can be observed that both soils have low organic matter content, low base exchange capacity, low to very low levels of $Ca^{2+}$, $Mg^{2+}$, $K^{+}$, $Na^{+}$ cations, as well as phosphorus (4). The analysis of the quantification of the number of spores indicated that in both soils there is an autochthonous AMF community with very high values of these structures, being much higher in Cabacú region than in Playa Duaba.

In previous studies, when quantifying the spore contents of autochthonous AMF from different Baracoa localities, cultivated with coconut, high levels of spores per 50 grams of soil were also found (Cane 2313.33 and La Perrera 1731.67) (4). The high levels of mycorrhizal propagules in the study sites could be related to their location within the buffer zone of the Alejandro de Humboldt National Park, which constitutes the largest remnant of the best preserved mountain ecosystems in Cuba, and the area of greatest endemism in the Antilles (23). On the other hand, these are undisturbed areas that have not received fertilizers, pesticides, or any other chemical product for a period of almost 20 years, which could provoke alterations in the soil microbiota (24).

**Table 1.** Characterization of the arable horizon (0-0.20 m) of soils existing in the areas where the experiment was conducted

| Locality | Soil | pH | MO | $P_2O_5$ | Ca | Mg | K | Na | CCB | AMF spores |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ($H_2O$) | (g. kg$^{-1}$) | (mg kg$^{-1}$) | | (cmolc kg$^{-1}$) | | | | per 50g soil |
| Playa Duaba | ARh | 7.5 | 23.7 | 265 | 7.5 | 3.5 | 0.09 | 0.08 | 11.2 | 923.33 |
| Cabacú | GFLh | 7.6 | 25.5 | 57.2 | 16 | 11.5 | 0.03 | 0.21 | 27.7 | 2676.67 |

Methods: pH ($H_2O$), organic matter (OM) (Wakley-Black), assimilable phosphorus ($P_2O_5$) (Oniani, $H_2SO_4$ 1N), potassium and other exchangeable cations (Maslova) ($NH_4Ac$ pH 7) and Cationic Exchange Capacity (CEC) by sum of cations (17). AMF spores (18). Haplic Arenosol soil (ARh), haplic fluvic Gleysol (GFLh)

In coconut plantations in other geographical areas, native populations of AMF have also been studied in which high contents of their propagules have been found, such as those carried out in the Yucatan Peninsula, where a great diversity of families and genera was found, with a greater relative abundance of Glomeraceae (2), the family to which the strains used in this study belong.

Spore contents quantified in the present study were similar to those found in other soils, such as those belonging to the San Ubaldo-Sabanalamar Floristic Reserve in Pinar del Río province (25). These areas have sandy soils with low organic matter content and low cationic exchange capacity, in which between 2000 and levels higher than 5000 spores per 100 g of soil have been reported in semi-natural and covered savanna, both in dry and rainy periods. In Ciénaga de Zapata Biosphere Reserve, in swamp forests in the areas of Pálpite and Playa Máquina, high levels of these structures have also been reported, from 3000-7000 spores per 100 g of soil (26). These authors demonstrated that levels of propagules in the soil are favored by the rainy period. The rainfall regime could have had an influence on the conditions of the present experiment, considering that it was developed in areas of high rainfall well distributed throughout the year.

In data analysis to determine year effect, no interaction was found, so we worked with the mean of the years studied for each of the variables evaluated. Table 2 shows the analysis of the influence of the three AMF strains in combination with different levels of organic fertilizer on the indicators of vegetative development, in which a differential response was observed in the AMF-organic fertilizer level interactions. Regarding the Germination Speed Index, the treatments did not show significant differences in any of the locations studied, since at 120 das, similar levels were obtained in all variants for each location.

When analyzing the growth indicators of height, stem diameter and number of leaves, it was observed that in Playa Duaba, where the soil is ARh, the best results were achieved with the AMF strain *G. cubense*, combined with the lowest levels of organic fertilizer (10:1:1 and 4:1:1:1), which showed statistical similarity with the S:H:FC 1:1:1 control. Compared to the NPK 100 % control, they only showed statistical similarity in height and number of leaves.

In the Cabacú nursery, which has GFLh soil, the highest values were reached with the *R. irregularis* strain, but in this case combined with S:H:FC 4:1:1 and 2:1:1:1 levels, which showed statistical similarity in height and number of leaves with the organic fertilizer control. When analyzing the influence of AMF strains in this soil, a homogeneous response was observed between *G. cubense* and *R. irregularis* combined with the three levels of S:H:FC in relation to stem diameter and number of leaves, which exceeded the controls, while the response with *F. mosseae* was less uniform. In general, higher levels of vegetative development indicators were achieved at Playa Duaba than at Cabacú.

Figure 1 shows the results of indicator quantification of mycorrhizal establishment in coconut plants ecotype "Indio Verde-1", inoculated with AMF, grown in three levels of substrate, on both soils. In relation to inoculated treatments, a differential response of AMF strains was observed in the two soils studied, with *G. cubense* being the one that reached the highest levels in ARh, while *R. irregularis* was the best strain for GFLh.

Regarding % C (Figure 1A and D), no treatment exceeded the organic fertilizer control in any of the soils, however, for % IC (Figure 1B and E) in ARh soil, the strain *G. cubense*, combined with S:H:FC 10:1:1 and 4:1:1:1, showed statistical similarity with this control, while in GFLh, the three AMF strains combined with the different levels of S:H:FC, reached similar results.

The joint analysis of mycorrhization indicators (% C and % IC) allows a more complete evaluation, since % C corresponds to the number of roots with the presence of some of the fungal structures, while % IC quantifies the level of these structures within roots, among which are the arbuscules, in which the bidirectional exchange of nutrients and photosynthates between the plant and the fungus takes place (27). Considering this analysis, *G. cubense* is corroborated as the best AMF strain for ARh soil, whereas the three AMF strains show similar results for GFLh.

Spores are the resistance structures, which allow the permanence in time and dissemination of the fungi. The analysis of their quantification (Figure 1C and F) showed that in the ARh soil the existing autochthonous strains turn out to be more competitive than the inoculated ones for their permanence in the soil, which is to be expected since they have high levels of spore contents (923.33 spores per 50 gram of soil), however, in GFLh, despite having higher autochthonous AMF contents than ARh (1676.67 spores per 50 gram of soil), the strain *G. cubense* with S:H:FC 4:1:1 and 2:1:1:1 and *G. irregularis* with the three levels of S:H:FC, show statistical similarity with the organic fertilizer control and the soil.

**Table 2.** Germination speed index, height, diameter at the stem base and number of leaves of seedlings of coconut palm ecotype Indio Verde-1, inoculated with AMF in three levels of substrate on the soils haplic Arenosol and haplic fluvic Gleysol

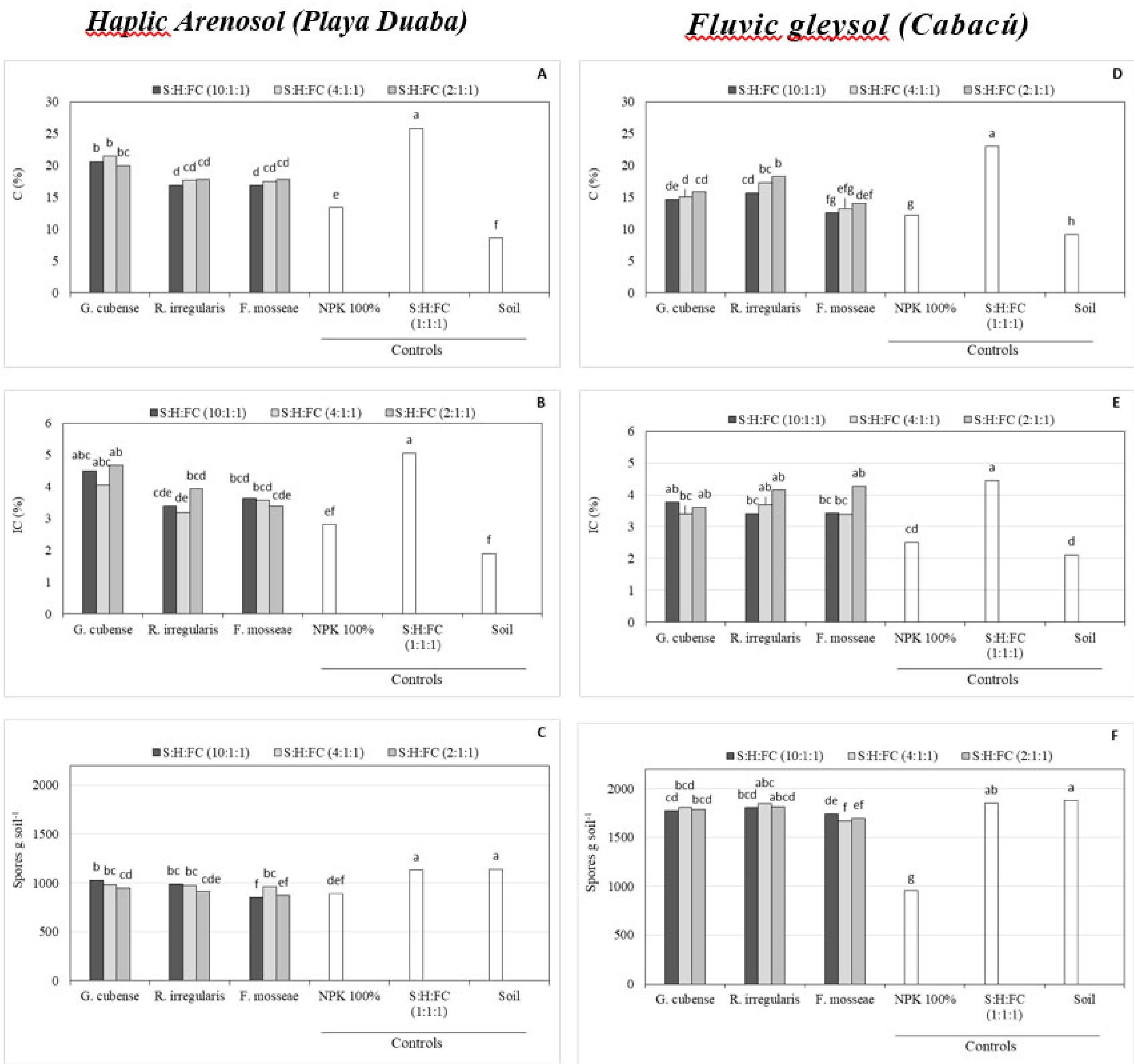
| *AMF* | *Levels of OM* | | *Height* (cm) | | *Stem diameter* (cm) | | *Leaf number* | |
|---|---|---|---|---|---|---|---|---|
| | | | **Haplic Arenosol soil (Playa Duaba)** | | | | | |
| *G. cubense* | S:H:FC (10:1:1) | 0.39 | 96.71 | bc | 3.11 | a | 3.00 | a |
| | S:H:FC (4:1:1) | 0.42 | 95.92 | bc | 3.10 | a | 2.82 | abc |
| | S:H:FC (2:1:1) | 0.42 | 94.21 | cd | 3.08 | ab | 2.82 | abc |
| *R. irregularis* | S:H:FC (10:1:1) | 0.39 | 89.46 | def | 2.83 | bc | 2.67 | cd |
| | S:H:FC (4:1:1) | 0.41 | 88.60 | def | 2.69 | cd | 2.58 | d |
| | S:H:FC (2:1:1) | 0.40 | 87.47 | ef | 2.53 | d | 2.58 | d |
| *F. mosseae* | S:H:FC (10:1:1) | 0.39 | 93.02 | cde | 3.04 | ab | 2.73 | bcd |
| | S:H:FC (4:1:1) | 0.40 | 91.33 | cde | 3.00 | ab | 2.71 | cd |
| | S:H:FC (2:1:1) | 0.38 | 89.21 | def | 2.75 | cd | 2.62 | cd |
| NPK 100% | | 0.43 | 104.20 | a | 2.83 | bc | 2.82 | abc |
| S:H:FC(1:1:1) | | 0.45 | 101.03 | ab | 2.92 | abc | 2.91 | ab |
| soil | | 0.42 | 83.06 | f | 2.67 | cd | 2.69 | cd |
| SEx | | 0.02 ns | 2.08 | | 0.08 | | 0.02 | |
| | | | **haplic fluvic Gleysol (Cabacú)** | | | | | |
| *G. cubense* | S:H:FC (10:1:1) | 0.27 | 88.11 | c | 2.75 | ab | 2.71 | abc |
| | S:H:FC (4:1:1) | 0.26 | 91.03 | bc | 2.79 | a | 2.78 | abc |
| | S:H:FC (2:1:1) | 0.26 | 90.20 | c | 2.77 | ab | 2.71 | abc |
| *R. irregularis* | S:H:FC (10:1:1) | 0.27 | 91.03 | bc | 2.80 | a | 2.78 | abc |
| | S:H:FC (4:1:1) | 0.26 | 97.08 | ab | 2.84 | a | 2.84 | ab |
| | S:H:FC (2:1:1) | 0.26 | 97.71 | a | 2.83 | a | 2.89 | a |
| *F. mosseae* | S:H:FC (10:1:1) | 0.26 | 85.77 | c | 2.71 | abc | 2.67 | bc |
| | S:H:FC (4:1:1) | 0.27 | 86.56 | c | 2.58 | abcd | 2.76 | abc |
| | S:H:FC (2:1:1) | 0.26 | 87.97 | c | 2.52 | bcd | 2.76 | abc |
| NPK 100% | | 0.26 | 97.62 | a | 2.44 | d | 2.62 | c |
| S:H:FC(1:1:1) | | 0.26 | 100.92 | a | 2.49 | cd | 2.89 | a |
| soil | | 0.25 | 76.76 | d | 2.14 | e | 2.6 | c |
| SE x | | 0.01 ns | 2.08 | | 0.08 | | 0.02 | |

Means with different letters for the same soil differ from each other, according to Duncan's Multiple Range Test (P≥0.05) GVI: germination speed index, S-H-FC: soil:earthworm humus:coconut fiber. SE x: standard error of the mean

One aspect that is interesting to note is the high levels of native AMF propagules in the soils under study, which would lead one to believe that it is difficult for the applied strains to become established. It would be expected that with high levels of autochthonous AMF, no response to inoculation would be obtained, because competition for nutrients would be generated, where the autochthonous species would have a better result, taking into account the level of adaptation to the edaphic conditions present. However, the results found in this work could be due to the fact that, when preparing the substrate mixture consisting of soil: earthworm humus and coconut fiber, at different concentrations of organic fertilizer, there was a decrease in the levels of propagules existing in the soils studied, taking into account that the soil constitutes only a percentage of the total mixture volume, an effect that was reflected in the responses of plants.

The differential results obtained in relation to AMF establishment indicators in both soils may be due to the fact that the ARh soil has high P contents, which can inhibit the establishment of the introduced strains, unlike the autochthonous strains that are adapted to these contents. On the other hand, in the GFLh, these P levels are lower, which allowed a better establishment of the inoculated strains. The establishment of arbuscular mycorrhizal symbiosis is regulated by different factors, among which are the P concentrations in the soil (5,28), the balance of which is regulated according to the plant's P needs (29). In a study of the influence of different levels of $KH_2PO_4$ it was found that, at concentrations between 20 and 200 μM, high percentages of colonization were obtained in stevia, being the concentration of 200 the most favorable for the formation of arbuscules; however, both indicators were significantly reduced at 500 and 1000 μM of $KH_2PO_4$, so it was concluded that low concentrations of P induce high percentages of colonization (5).

In both soils it was observed that fertility conditions need to be improved to achieve the establishment of AMF in the roots of coconut seedlings, which can be affirmed, given that the levels of mycorrhization indicators, such as the percentage of colonization and colonization intensity, reached similar or lower levels than those obtained in the S:H:FC control (1:1:1:1).




Means with different letters for the same soil differ from each other, according to Duncan's Multiple Range Test (P0,05). S:H:FC: soil:worm humus:coconut fiber. SEx: standard error of the mean

**Figure 1.** Colonization (C %), intensity of colonization (IC %) and quantification of the number of spores on coconut plants ecotype "Indio Verde-1" inoculated with AMF, grown in three substrate levels on haplic Arenosol and fulvic Gleysol soils

The organic fertilizer may have improved the physical properties of the soils with which it was used, allowing greater availability of nutrients and thus producing better plant development. In a study with passion fruit (*Passiflora edulis*), using coconut mesocarp powder in the substrate and the AMF *Acaulospora longula*, it was concluded that the addition of the organic fertilizer favored mycorrhization, with a positive effect on vegetative development and physiological indicators of the plants (30).

AMF increase the volume of soil to be explored by roots through a network of interconnected hyphae, with which they increase the uptake of nutrients, including phosphorus, nitrogen, zinc, iron, as well as sugars and water (7). These play a critical role in natural and cultivated ecosystems with nutrient limitations, such as nitrogen and phosphorus (31,32). However, when NPK 100 % is applied, the plant will only have at its disposal the nutrients incorporated through fertilization and those found in the soil.

Other authors, studying the influence of AMF on coconut seedlings, found a positive effect of inoculation, with increases in the development of primary, secondary, tertiary and quaternary roots, which improved the efficiency of water and nutrient absorption and induced increases in the perimeter of the base of the stem and the dry weight of the aerial part (33).

## CONCLUSIONS

With the results obtained, it is concluded that there was a differential response of the interaction AMF-organic fertilizer concentrations in soils studied, obtaining the best results, in general, with the lowest levels of organic matter (S:H:FC 10:1:1 and 4:1:1:1). In the ARh, corresponding to the Playa Duaba nursery, only with *G. cubense* similar results to the controls were achieved, while in the GHh soil, Cabacú nursery, similar levels to the controls were achieved with the three AMF strains. These alternatives favored a 30-day reduction in the period of permanence in the nursery, with an increase in the value/cost ratio in the production process. Seedlings obtained showed greater survival and growth than those produced by conventional management when planted in two agroecosystems, which demonstrated the effectiveness of the proposed agroecological management. Considering the low availability of organic fertilizers in the localities where the nurseries are located, these alternatives are very useful because they allow the substitution of 75 % of the organic fertilizer.

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