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Original article

Benefits of local sources of nutrients and mycorrhizal inoculants in micrografts of *Theobroma cacao* L

Beneficios de fuentes locales de nutrientes e inoculantes micorrízicos en microinjertos de *Theobroma cacao* L

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ABSTRACT: Cocoa requires more efficient technologies that are based on local sources of nutrients and enhancing biological activity. The work was developed through two experiments carried out during the period 2016-2019, in the Coffee and Cocoa Enterprise of Baracoa, Guantanamo province. Cacao and coconut husks and earthworm humus, all available to producers, were used as sources of organic fertilizers (OA). Three soil / OF ratios of 3:1, 5:1, 7:1 v/v were studied for each of them. Subsequently, the response to the mycorrhizal fungi strains *Glomus cubense* (INCAM-4) and *Rhizoglomus irregulare* (INCAM-11) was studied. The soil used was a brown loam with carbonates. A randomized block design with a factorial arrangement and four replications was used. Plant height, number of pairs of leaves, stem diameter, leaf area and percentage of mycorrhizal colonization were evaluated. The substrates composed of cocoa husk and earthworm humus with S/OF ratios of 5/1 and 3/1 presented the highest growth rates of the seedlings, with no differences between cocoa husk and earthworm humus. Coconut husk produced the smallest seedlings. The inoculation of the micro-graft with *R. irregulare* or *G. cubense* in cocoa husk or worm castings substrates in S/AO ratio (5:1) was effective, presenting better indicators in size and leaf area and a 44 % decrease in the amount of organic fertilizers in relation to the recommendation of the Technical Instructions of 3/1.

Key words: organic fertilizers, biofertilizers, mycorrhizal infection, seedlings.

RESUMEN: El cacao requiere de tecnologías de producción más eficientes que se basen en fuentes locales de nutrientes y potencien la actividad biológica. Se ejecutaron dos experimentos para optimizar la obtención de posturas vía microinjerto, durante el periodo 2016-2019 en la Empresa de Café y Cacao de Baracoa, provincia de Guantánamo. Se emplearon como fuentes de abonos orgánicos (AO) las cáscaras de cacao y coco y el humus de lombriz, todas con disponibilidad para los productores. Se estudiaron para cada una tres proporciones suelo/AO de 3:1, 5:1, 7:1 v/v. Con posterioridad se estudió la respuesta a las cepas de hongos micorrízicos arbusculares *Glomus cubense* (INCAM-4) y *Rhizoglomus irregulare* (INCAM-11). El suelo utilizado fue Pardo mullido carbonatado. Se utilizaron diseños de bloques al azar con cuatro réplicas. Se evaluaron la altura de las plantas, el número de pares de hojas, el diámetro del tallo, el área foliar y el porcentaje de colonización micorrízica. Los sustratos conformados con cáscara de cacao y humus de lombriz, en relación S/AO de 5/1 y 3/1, presentaron los mayores índices de crecimiento de las posturas, sin diferencias significativas entre estos. La cáscara de coco originó siempre las posturas más pequeñas. La inoculación del microinjerto con *R. irregulare* o *G. cubense* en sustratos de cáscara de cacao o humus de lombriz en relación S/AO (5:1) fueron efectivas, presentando mejores indicadores en tamaño y área foliar y disminución en 44 % de las cantidades de abonos orgánicos con relación a la recomendación del Instructivo Técnico de 3/1.

Palabras clave: cáscara de cacao, humus de lombriz, cáscara de coco, posturas, hongos micorrízicos arbusculares.

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INTRODUCTION

Cocoa is one of the most important agricultural exports for the country, with a wide range of derivatives destined for different branches of the industry. Its cultivation is mainly concentrated in some areas of the eastern region of Cuba, due to its edaphoclimatic characteristics with friable soils and high annual rainfall (1700-2500 mm), with plantations predominating in Baracoa municipality, in Guantánamo province, with about 68 % of these and 87 % of national production (1).

This crop has been propagated, ancestrally, by seed, but in the 20th century it began to be propagated vegetatively, through cuttings and grafts. Likewise, new methods have been proposed (2) to make its production more effective, fast and profitable; among these, the rooting of cuttings directly in the bag, micro-grafting and *in vitro* culture; however, its multiplication has been affected by the low quality of the substrates in which the seedlings are reproduced, producing trees of low productivity due to the origin and conformation of the substrate and its handling (3).

Nowadays, the sustainability of agricultural systems must promote the effective use and management of the natural resources of agroecosystems. In this sense, beneficial microorganisms are a vital component to mitigate climate change and achieve resilient agriculture. These include endophytic microorganisms, nitrogen-fixing bacteria, antagonistic microorganisms, efficient microorganisms, as well as other soil endophytes such as arbuscular mycorrhizal fungi (AMF) (4-7), making their use an economically attractive and ecologically acceptable solution to reduce the use of chemical and even organic fertilizers and improve the efficiency of natural resources (8-10).

Commonly in the production of seedlings in the country, nutrients are provided through applications of organic fertilizers, which, in addition, improve the physical characteristics of the substrates. The most commonly used sources of fertilizers have been worm humus, manures and composts (2, 11, 12). In the municipality of Baracoa, local organic sources include cocoa and coconut husks, which are used after going through natural decomposition processes (2, 11). The former, which represents 40 % of the organic matter produced in this municipality, is an easily decomposed material, with high moisture retention and its application improves the physical-chemical characteristics of the soil (13, 14); the latter, although not easily decomposed, is abundant and inexpensive (11). For both sources it is important to find efficient uses for them and to reduce possible sources of contamination associated with their accumulation.

For more than 20 years in Cuba, a series of investigations related to the use and management of AMF in different agroecosystems have been developed, whose results have provided important contributions in the use and management of this biofertilizer in different crops and its benefits (15, 16). Specifically in the production of seedlings, applications of efficient AMF strains have shown positive effects in crops such as banana (12), coffee (17) and avocado (18), guaranteeing optimal development and greater survival of seedlings in the transplant and requiring smaller amounts of organic fertilizer to make up the substrates than those indicated by the respective Technical Instructions of the crops.

Although cocoa is a mycotrophic crop (19, 20, 21), few studies have been carried out in Cuba on the response of cocoa to inoculation with efficient AMF strains. There is only one reported work done with cocoa seedlings obtained from hybrid seeds, evaluating two AMF strains and proportions of soil/cachaça as substrates (22) and obtaining the best results in the growth of seedlings and nutrient absorption, with the simple application of *Glomus cubense* (INCAM-4) or *Rhizoglomus irregulare* (INCAM-11).

Therefore, it is important to evaluate effects of various local sources of organic fertilizers (OA), soil/OA ratios and the inoculation of efficient AMF strains, whose integration could be the basis for improving the efficiency and feasibility of the technology of seedling production via micro-grafts.

MATERIALS AND METHODS

General experimental conditions

The work was developed through two experiments in the stage of production of seedlings of *Theobroma cacao* L, which were repeated for two years each, during the period 2016-2019, in the nursery of Coffee and Cocoa Company of Baracoa, located in Los Hoyos de Sabanilla, Baracoa municipality, Guantánamo province. The postures were developed in bags of 14.5 cm x 24 cm, with a capacity of 1.1 kg of substrate.

The soil used to make up the substrates corresponds to a carbonated mellow brown (23), some of whose chemical characteristics are reflected in Table 1.

The chemical analysis was carried out at the Soil Laboratory of Guantánamo province. The methods used were: pH-H_2O in the soil: solution ratio (1: 2.5) by the potentiometric method; the determination of organic matter by the Walkley-Black method (oxidation of C with $K_2Cr_2O_7$ 0.5 M in H_2SO_4 (18 M at 98 %) and titration with $FeNH_4$ SO_4 (0.25 M); determination of available P_2O_5 and K_2O by Machiguin's method (extractive solution of (NH4)2CO3 at a

Table 1. Chemical characteristics of the carbonated brown mellow soil

Type of soil	pH (H_2O)	OM (%)	P_2O_5	K_2O	K^+	Ca^{2+}	Mg^{2+}	CIB
			mg 100g^{-1}		cmol$_c$kg^{-1}			
Brown	7.25	3.6	2.14	30.32	0.64	31.5	11.8	44.3

CEC: Cations Exchange Capacity=∑ Changeable cations concentration of 10 g L^{-1}, pH 9.0) and titration with ascorbic acid; exchangeable cations by extraction 1/5 with NH_4Ac 1 M pH 7 and determination by complexometry (Ca and Mg) and flame photometry (K).

Micrograft management and care

Micrografting is a technique used to graft a small bud of a productive cultivar onto the hypocotyl of a seedling of a rootstock selected for its robustness (1). For the formation of the micrografts, cultivar UF-650 was used as rootstock and buds came from cultivar UF-654 (24). Both cultivars, recognized for their high productive potential, came from the Cocoa Germplasm Bank of the Baracoa Science and Technology Base Unit (UCTB).To obtain the seedlings, the following procedure was used: the seeds of the rootstock were spread in a pre-germinator with river sand, previously disinfected with formol solution (1 %) and then washed with abundant water. When the radicle reached 5 mm, they were sown in the bags, at the rate of one seed per bag. Micrografting was performed approximately 15 days later, when the seedling reached a height of 10 to 15 cm and before the cotyledon opened (1). Drip irrigation was used during the experimental period.

Sources of organic fertilizers used

Cocoa husk. Cocoa husks decomposed in wooden beds were used for a period of 6 months and according to the traditional method of farmers in Baracoa, which includes turning every 30 days and maintaining humidity between 40 and 60 % (2). The product presented a dark color and a lumpy texture, indicative of satisfactory decomposition.

Coconut husk. Due to its high percentage of cellulose and lignin (11, 25), coconut husks are slow to decompose naturally. A semi-decomposed material was used, deposited for 14 months in an open-air stowage. Subsequently, it was ground and the fibers were extracted. The material was of a bronw color and lumpy texture.

Worm humus. The worm humus used was made (26) in the worm humus production center of the "Paso de Cuba" Management Center, attached to the Baracoa Agroforestry and Coconut Enterprise. The vegetable residue used for its preparation was semi-decomposed coffee husk. The color of the humus was dark and well granulated.

Experiment 1. Evaluation of the sources of organic fertilizer and the S/AO ratio suitable for the production of cocoa seedlings by micro-grafting.

The experiment was developed between the months of May to August 2016 and 2017. The effect of three sources of organic fertilizer (cocoa and coconut husks and worm humus), all available to producers and three soil: organic fertilizer (S/AO) ratios of 3:1, 5:1 and 7:1 v/v, was evaluated using a randomized block design with factorial arrangement (3x3) and four replications, for a total of nine treatments. The S/AO ratio recommended by the Technical Instructions is 3:1, based on the use, indistinctly, as sources of organic fertilizer: earthworm humus, compost, manure, decomposed cocoa or coffee husks (1).

Treatments:

1. Soil: cocoa husk 3:1 v/v (production control)
2. Soil: cocoa husk 5:1v/v
3. Soil: cocoa husk 7:1v/v
4. Soil: earthworm humus 3:1v/v (production control)
5. Soil: earthworm humus 5:1v/v
6. Soil: earthworm humus 7:1v/v
7. Soil: coconut husk 3:1v/v (production control)
8. Soil: coconut husks 5:1v/v
9. Soil: coconut husks 7:1v/v

Experiment 2. Response of cocoa seedlings obtained by micrografting to inoculation with AMF strains.

The experiment was developed in the same period (May to August) and during 2018 and 2019. A randomized block design with six treatments and four replicates was used. The treatments consisted of four combinations from cocoa husk or worm humus always in the S/AO ratio of 5/1 in the presence of simple inoculation with AMF strains: INCAM-4 or INCAM-11 and two treatments of both sources in the S/AO ratio of 3/1 and without inoculation (production controls). The experimental plots consisted of 50 stands of which 25 were evaluated. The same type of bag and irrigation management were used as in the previous experiment.

Treatments:

1. Soil: cocoa husk 5:1v/v + INCAM-11.
2. Soil: earthworm humus 5:1v/v + INCAM-11
3. Soil: cocoa husk 5:1v/v+ INCAM- 4
4. Soil: vermicompost humus 5:1v/v+ INCAM-4
5. Soil: cocoa husk 3:1v/v uninoculated (production control)
6. Soil: worm humus 3:1v/v uninoculated (production control)

The arbuscular mycorrhizal fungi (AMF) strains used were *Glomus cubense* (27)/INCAM-4, DAOM241198 and *Rhizoglomus irregulare* Syn. *Rhizophagus irregularis* (28) / INCAM-11, DAOM711363, from the National Institute of Agricultural Sciences (INCA) of Cuba. Both were formulated as simple solid inoculants (15), with more than 60 spores per gram of product and an undetermined amount of mycelia and infective rootlets.

The inoculation of each strain was carried out by coating the seeds of the rootstock with a mixture of the inoculant and water (15). For the preparation of the mixture, the inoculant was applied at a ratio of 10 % in relation to the fresh weight of the seed. Inoculated seeds were placed in the pre-germinator and the procedure was similar to experiment 1 in relation to the management of the rootstock and the micro-grafting.

Evaluations carried out at four months.

1. Plant height (cm): a tape measure was used, measured from the root collar to the terminal bud.

Number of leaves per plant (unit).

2. Stem diameter (cm): measured below the cotyledonal scar, with a caliper with a precision of 0.05 cm.

3. Leaf area (cm^2): it was estimated from the length and width of the leaves, using the following formula (29).

$$AF = 0{,}6766(LxA) - 1{,}843$$

4. Percentage of mycorrhizal colonization: Fine root samples were taken from eight plants per plot, located in the central part. The roots were stained with blue ink (30) and evaluated by the intercept method (31).

Statistical procedure. The experimental results were processed according to the experimental design and arrangements used, and the corresponding analysis of variance was performed with the Statgraphics program version 5.1, in a Windows environment. The mycorrhizal colonization data (percentage of colonization) were transformed by Arc. sen√x. When the analysis of variance was significant, Duncan's multiple range comparison test for p<0.05 % was applied as a criterion for comparison between treatment means.

RESULTS

Experiment 1: Evaluation of the sources of organic fertilizer (OA) and the S/AO ratio suitable for the production of cocoa seedlings by micrografting.

In relation to plant height and stem diameter, the best results, in both years, were obtained in the cocoa husk and worm humus treatments in the 3:1 and 5:1 ratios, with no significant differences between them (p<0.05). The 5:1 ratio was always higher (p<0.05) than the 7:1, while the 3:1 was significantly higher than the 7:1, only in 2017 (Table 2).

The number of leaves presented a similar behavior with the highest values when using cocoa husk or vermicompost in the 5/1 and 3/1 ratios; however, significant effects were only found in 2016. In that year, the 5/1 and 3/1 ratios presented similar values, although only the 5/1 ratio originated a greater number of leaves (p<0.05) than the 7/1. After four months, the micrografts reached the optimal quality values of a cocoa stand, with diameters between 7.50-8.57 mm and 11-12 leaves (1).

The coconut husk treatments in the three proportions presented the lowest values (p<0.05) in all variables and years, with no significant differences among them. These treatments always produced lower values (p<0.05) compared to those obtained with cocoa husk or worm castings in the 5/1 and 3/1 ratios and without significant differences with those obtained in the 7/1 ratio.

Figure 1 shows the effects of the different sources and proportions studied on the leaf area of the seedlings at four months of age and in both years. The treatments where cocoa husk and earthworm humus were applied in 3:1 and 5:1 proportions, produced seedlings with a leaf area between 1215 cm2 and 1301 cm2 (Figure 1), without significant differences between these values (p<0.05) and always higher than the rest of the treatments studied.

The lowest leaf area indexes (p<0.05) were reached in the seedlings produced in the coconut husk substrate in any of the proportions, as well as when the 7/1 ratio was used, with no significant differences between these treatments.

Although the micro-grafts achieved the highest growth indicators with S/AO ratios of 5/1 and 3/1, from cocoa husk and worm castings sources, the lower amounts of organic fertilizer used in 5/1 defined their use with both sources in experiment 2.

Experiment 2. Response of cocoa seedlings obtained by micrografting to inoculation with AMF strains.

Inoculation of the seedlings with *G. cubense* or *R. irregulare* in substrates prepared with cocoa husk or worm castings, both with a 5:1 S/AO ratio, presented the highest height values in both years, without differences between them (Table 3) and significantly higher (p<0.05) than the non-inoculated treatments, consisting of substrates with an S/AO ratio of 3/1 and corresponding to the production controls (1). Both stem diameter and number of leaves showed a similar behavior to the previous one, so

Table 2. Effect of sources and proportion of organic manure on some indicators of cocoa seedling growth during 2016-2017

Treatments	Height plant (cm)		Stem diameter (mm)		Leaf number (u)	
	2016	2017	2016	2017	2016	2017
3:1Soil/CC_1	29.36 ab	30.83 a	7.61ab	7.46 a	11.7 ab	10.58
5:1 Soil/CC_1	29.54 a	31.33 a	8.23 a	7.66 a	12.4 a	11.72
7:1 Soil/CC_1	24.80 bc	26.70 bc	7.10 bc	6.27 b	10.6 bc	10.28
3:1 Soil/HL	28.34 ab	29.40 a	7.01 bc	7.56 a	11.7 ab	11.14
5:1 Soil/HL	30.97 a	30.44 a	8.57 a	7.50 a	12.0 ab	12.00
7:1 Soil/HL	24.66 bc	27.06 bc	6.84 bc	6.43 b	10.5 bc	10.86
3:1 Soil/CC_2	23.47 c	23.73 c	6.67 bc	6.50 b	10.5 bc	10.28
5:1 Soil/CC_2	23.63 c	24.74 c	6.49 c	6.67 b	10.2 bc	10.28
7:1 Soil/CC_2	23.16c	24.07 c	6.27 c	6.44 b	9.70 c	10.28
Se $\bar{x}$	1.06*	1.03*	0.31*	0.30*	0.30*	0.30 ns

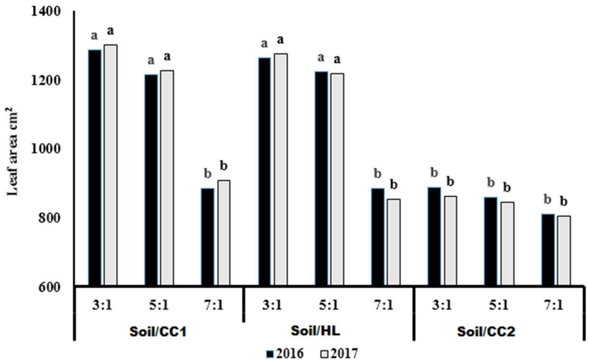
CC1: cocoa husk (3:1.5:1.7:1 v/v), HL: earthworm humus (3:1.5:1.7:1 v/v), CC2: coconut husk (3:1.5:1.7:1 v/v).

* Means with different letters in the same column differ from each other according to Duncan's test for p<0.05

that plants inoculated with any of the strains and substrates with a 5:1 v/v ratio were superior to the production controls.

Regarding leaf area, inoculation significantly increased this variable in both years, in comparison with the non-inoculated treatments and without significant differences between the responses to the inoculated strains; likewise, no significant effect of the organic fertilizer sources factor was found. The increases obtained in both years when inoculating *R. irregulare* were between 19 and 20 %, similar ($p<0.05$) to those achieved with *G. cubense* between 14 and 16 %, always with respect to the non-inoculated controls (Figure 2).

Likewise, the percentage of mycorrhizal colonization (Table 4) reflected a positive response ($p<0.05$) to mycorrhizal inoculation, which was similar in both years, with values between 33 and 38 %, with no differences between strains or between organic fertilizer sources.



CC1: cocoa husk; HL: earthworm humus; CC2: coconut husk. Means with different letters in each year differ from each other according to Duncan's test for $p<0.05$. Se $\bar{x}$: 66.99* in 2016 and 54.17* in 2017

Figure 1. Effect of different substrates and S/AO ratios on leaf area of 4-month-old cocoa seedlings obtained by micrografting, during 2016-2017

DISCUSSION

In general, for two years, the feasibility of the application of local organic sources of cocoa husk and earthworm humus in mixtures with carbonated mulched brown soil, for the production of cocoa seedlings by micro-grafts, has been demonstrated. This ratio can be reduced to 5:1 and allows a considerable saving of 44% of organic fertilizer, compared to the 3/1 ratio recommended in the Technical Instructions (1) and, therefore, this ratio of 5/1 is the one selected to carry out experiment 2.

This result could be explained by taking into account the nutrient contents provided by cocoa shells, in the order of 0.7 to 1 % nitrogen and 1.3 to 1.7 % potassium (13, 32, 33), in addition to the fact that their organic matter and porosity contents (32) increase moisture retention, biological activity and improve the physicochemical characteristics of the soil (13, 32, 33).

The use of an organic fertilizer system that takes into account the use of earthworm humus also ensures favorable conditions for the germination and subsequent growth of plants, since the addition of earthworm humus to soils and substrates considerably increases the growth and productivity of a large number of crops, by significantly improving their physical, chemical and biological properties (26, 34).

In relation to the use of decomposed cocoa husk, the results obtained corroborate its equivalence with earthworm humus to form substrates and it is also a recommended practice for cocoa producers (1). In the case of Baracoa, the use of decomposed cocoa shells as fertilizer is feasible, since it represents 40 % of the organic matter produced in the municipality and with it, quality postures are achieved. Nevertheless, it may be important to study, in later works, the use of manure to accelerate the decomposition speed of the cocoa husk (35) or to produce worm humus based on this material and be able to manage more efficiently the high volumes of this residue that are generated, since the husk represents around 70 % of the harvested fruit (32, 36).

Regarding coconut husk, although Guantánamo has 80 % of the country's coconut production and the husk is an abundant waste, since it constitutes approximately 55 % of the fruit mass (11); However, it is a material that is difficult to decompose due to its high lignin and cellulose content (11, 25), which may have influenced the poor growth and low quality of the cocoa seedlings obtained from it, compared to substrates made from worm castings or cocoa husks.

Table 3. Effect of AMF strain inoculation on the growth of cocoa seedlings obtained by micrografting

Treatments	Height plant (cm)		Stem diameter (mm)		Leaf number	
	2018	2019	2018	2019	2018	2019
5:1 Soil/CC1+ INCAM-11	32.71 a	30.14 a	7.90 a	6.97a	14.93a	14.90a
5:1 Soil /HL+ INCAM-11	31.25 a	30.43 a	7.62 a	6.87a	14.98a	15.02a
5:1 Soil /CC1+ INCAM-4	31.94 a	31.14 a	7.55 a	7.03 a	15.15 a	15.12 a
5:1 Soil /HL+ INCAM-4	31.71 a	31.57 a	7.54 a	6.80 a	15.10 a	14.95 a
3:1 Soil /CC1 (control)	26.70 b	26.57 b	6.35 b	5.84 b	12.72b	12.10b
3:1 Soil /HL (control)	28.14 b	27.93 b	6.40 b	5.98 b	12.80b	12.66b
Se $\bar{x}$	1.02*	1.17*	0.30*	0.21*	0.30 *	0.32*

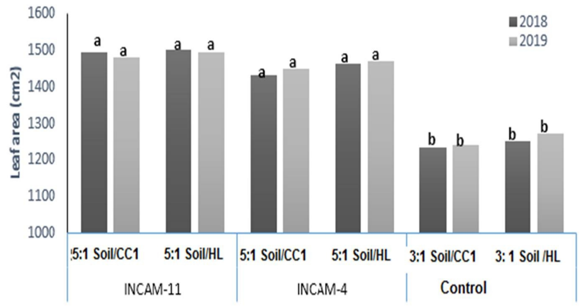
CC1: cocoa husk; HL: worm castings. INCAM-4: *G. cubense* and INCAM-11: *R. irregulare*. 3:1 S/AO ratio recommended by the Technical Instructions of the crop (1). Means with different letters in the same column differ from each other according to Duncan's test for p<0,05. * ANOVA significant for p<0.05

Therefore, despite its abundance, it should not be used as a semi-composted material to guarantee the supply of nutrients in the production of cocoa seedlings. However, its previous composting seems to improve, significantly, its characteristics as organic fertilizer (25) and can be one of the solutions to process the high amount of existing residues (11).

The micro-graft responded positively to the inoculation of either of the two AMF strains used, increasing the benefits achieved in the first experiment, since not only can the amount of organic fertilizer in the substrate be reduced, but also the postures present better growth in all the evaluated indexes.

The most detailed information in Cuba on the use of AMF in nurseries of perennial plants are the works with coffee plants (17), developed in the Sierra Maestra and Guamuhaya massifs, on a group of soils of very varied fertility conditions (Alitic, Fersialitic and Pardos). One of the most important results was the positive and consistent response found, achieving in all the soils studied certain combinations of AMF strain and soil/organic fertilizer ratio, with significant increases in growth and leaf area, as well as a decrease in the amounts of organic fertilizers needed to be applied to the substrate. In these studies, the strains with the greatest response and the S/AO ratios suitable for the greatest effectiveness of the inoculation varied with the type of soil. To the extent that the soil studied presented greater fertility, the quantities of organic fertilizer needed in the substrate to allow effective mycorrhization decreased, and vice versa.

The species of the Glomeracea family have a wide range of functional distribution with predominance in ecosystems of high and medium fertility, where they are extremely efficient and competitive. The results obtained in Cuba, from using simple inoculants of "generalist" strains with plant species in a wide range of crops and soil types (15, 37) have allowed recommending the application of INCAM-11 (*R. irregulare*) to crops in Brown cabonate, Calcimorphic humic, Vertic and even Red ferrallitic soils, provided that the pH-H_2O ≥7. The INCAM-4 application (*G. cubense*) is recommended in soils whose pH is in the range of 5.8 to 7.2, with an overlapping zone of effectiveness of both strains between pH 7 and 7.2. Therefore, the results found in these soils with pH-H_2O 7.25, with positive and similar responses to inoculation, both INCAM-11 and INCAM-4, seem to be consistent with the established recommendation criteria and allow cocoa to be included in the group of crops that meet them.



CC1: cocoa husk; HL: earthworm humus. 3:1 Soil/AO, ratio recommended by Technical Instructions for Cocoa Cultivation (1). INCAM-4: *Glomus cubense* and INCAM-11: *Rhizoglomus irregulare*. Means with different letters in each year differ from each other, according to Duncan's test for p<0.05. Se x̄: 57.07* in 2018 and 53.88* in 2019

Figure 2. Effect of inoculation with AMF strains and different substrates on leaf area of cocoa seedlings obtained by micrografting, during 2018-2019

Although for the cultivation of cocoa, in brown soils of Baracoa with pH of 7.25, *R. irregulare* and *G. cubense* strains can be used indistinctly, taking into account the importance of the chemical reaction of the soil in the effectiveness of these strains (15), it is recommended, for the effective use of these inoculants in other soils of the region, to determine the pH-H_2O and select the strain in question according to this.

The positive effects of the inoculation on the colonization percentages were similar to those obtained in the growth indicators, showing that the inoculation was effective; mycorrhizal functioning was increased and, consequently, the inoculated plants presented better growth rates, which has been observed in different crops when applying these mycorrhizal inoculants (16, 17, 38, 39). The colonization

Table 4. Effect of AMF strain inoculation on mycorrhizal colonization (%) of cocoa micrografts. Years 2018 and 2019

Treatments	Colonization (%)	
	2018	2019
5:1 Soil/CC1+ INCAM-11	38.00a	37.22 a
5:1 Soil/HL+ INCAM-11	37.00 a	37.00 a
5:1 Soil/CC1+ INCAM-4	34.33 a	33.00 a
5:1 Soil/HL+ INCAM-4	33.00 a	34.00 a
3:1 Soil/CC1 (Control)	16.05 b	17.24 b
3:1 Soil/HL (Control)	18.17 b	19.14 b
Se x̄	1.11*	1.15*

CC1: cocoa husk; HL: earthworm humus. INCAM-4: G. cubense and INCAM-11: *R. irregulare*. 3:1 Soil/AO, ratio recommended by the Technical Instructions for Cocoa Cultivation (1). Means with different letters in the same column differ from each other according to Duncan's test for p<0.05

percentages obtained in the inoculated treatments were similar to those found when applying mycorrhizal inoculants in cocoa nurseries developed under other experimental conditions (19, 40), in which the inoculated plants presented values of up to 40 % colonization at 120 days, while the controls were of the order of 14 %. Although mycorrhizal colonization in the non-inoculated treatments allows establishing the presence of resident strains in the natural soil, both growth and colonization percentages were much lower compared to when the plants were inoculated, demonstrating the effectiveness of inoculation in cocoa under these conditions species of the Glomeracea family have a wide range of functional distribution with predominance in ecosystems of high and medium fertility, where they are extremely efficient and competitive. The results obtained in Cuba, from using simple inoculants of "generalist" strains with plant species in a wide range of crops and soil types (15, 37) have allowed recommending the application of INCAM-11 (*R. irregulare*) to crops in *Brown cabonate, Calcimorphic humic Vertic* and even Rd Red ferrallitic soils, provided that the pH-H_2O ≥7. The application of INCAM-4 (*G. cubense*) is recommended in soils whose pH is in the range of 5.8 to 7.2, with an overlapping zone of effectiveness of both strains between pH 7 and 7.2. Therefore, the results found in these soils with pH-H_2O 7.25, with positive and similar responses to inoculation, both INCAM-11 and INCAM-4, seem to be consistent with the established recommendation criteria and allow cocoa to be included in the group of crops that meet them.

Although for cocoa cultivation, in the brown soils of Baracoa with pH of 7.25, *R. irregulare* and *G. cubense* strains can be used indistinctly, taking into account the importance of the chemical reaction of the soil in the effectiveness of these strains (15), it is recommended, for the effective use of these inoculants in other soils of the region, to determine the pH-H_2O and select the strain in question according to this.

The positive effects of the inoculation on the colonization percentages were similar to those obtained in the growth indicators, showing that the inoculation was effective; mycorrhizal functioning was increased and, consequently, the inoculated plants presented better growth rates, which has been observed in different crops when applying these mycorrhizal inoculants (16, 17, 38, 39). The colonization percentages obtained in the inoculated treatments were similar to those found when applying mycorrhizal inoculants in cocoa nurseries developed under other experimental conditions (19, 40), in which the inoculated plants presented values of up to 40 % colonization at 120 days, while the inoculated plants presented values of up to 40 % colonization after 120 days (19, 40) in which the inoculated plants showed values of up to 40 % colonization at 120 days, while the controls showed values of about 14 %. Although mycorrhizal colonization in the non-inoculated treatments allows establishing the presence of resident strains in the natural soil, both growth and colonization percentages were much lower compared to when the plants were inoculated, demonstrating the effectiveness of inoculation in cocoa under these conditions.

CONCLUSIONS

The inoculation of the cocoa micro-graft with AMF *R. irregulare* or *G. cubense* strains is effective in cocoa husk or worm humus substrates, with S/AO ratio (5:1) in carbonated brown mellow soil, presenting better indicators in size and leaf area of the seedlings and a 44 % decrease in the amount of organic fertilizers, in relation to the recommendation of the Technical Instructions for Cocoa Cultivation to use an S/AO ratio of 3/1.

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