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FITOMAS-E AND ECOMIC® APPLICATION FOR REDUCTION OF MINERAL FERTILIZER CONSUMPTION IN PRODUCTION OF COFFEE TREE SEEDLINGS

Aplicación de FitoMas-E y EcoMic® para la reducción del consumo de fertilizante mineral en la producción de posturas de cafeto

Luperio Barroso Frómeta[1]✉, Maikel Abad Michel[2], Pedro Rodríguez Hernández[3] and Eduardo Jerez Mompié[3]

ABSTRACT. At the coffee nursery of Puriales Business Basic Unit, San Antonio del Sur municipality, Guantánamo province, were developed three experiments during three campaigns of seedlings production (April-September/2012, October/2012-March/ 2013, April-September/2013). The objective was evaluating the FitoMas-E and EcoMic® application for reduction of mineral fertilizer consumption in coffee tree seedlings production with adequate agricultural quality. The treatments by stage were: dose among 0,5 and 2,0 L ha^{-1} of FitoMas-E; two organic substrate with the best FitoMas-E dose and simple and combined mycorrhizal; proportional reductions of the mineral fertilizer from 100 % to 25 % with mycorrhizal and FitoMas-E combination. A completely randomized design in parcels of 1,44 m^2, and samples of ten plants for treatments was used. Simple ANOVA for experiments 1 and 3 and double ANOVA for experiment 2 were applied. The results showed the phytostimulant effect of FitoMas-E in the growth and development of coffee tree seedlings. The application of 1 L ha^{-1} of FitoMas-E is the best-suitable for the evaluated growth variables (dry matter and foliar area). In the nursery final phase, the best behaviour was in the plants with combined application of mycorrhizal and FitoMas-E in all experiments, in coffee pulp as substrate. This combination allows the reduction until a 25 % the mineral fertilizer, with higher results compared to the application of 100 % of this fertilizer.

Key words: coffee, nursery, mycorrhizal, FitoMas-E, fertilizer

RESUMEN. En el vivero de cafeto de la Unidad Empresarial Básica de Puriales, municipio San Antonio del Sur, provincia Guantánamo, se desarrollaron tres experimentos durante tres campañas de producción de posturas (abril-septiembre/2012, octubre/2012- marzo/2013, abril-septiembre/2013), con el objetivo de evaluar la aplicación de FitoMas-E y EcoMic®, para la reducción del consumo de fertilizante mineral en la producción de posturas de cafeto con adecuada calidad agrícola. Los tratamientos por etapa fueron: dosis entre 0,5 y 2,0 L ha^{-1} de FitoMas-E, dos sustratos orgánicos con la mejor dosis de FitoMas-E y la micorriza simple y combinada, reducciones proporcionales del fertilizante mineral desde un 100 % hasta el 25 % con la combinación de micorriza y FitoMas-E. Se utilizó un diseño completamente aleatorizado en parcelas de 1,44 m^2, y muestras de diez plantas por tratamiento. Se aplicó ANOVA simple, para los experimentos 1 y 3 y ANOVA doble para el experimento 2. Los resultados evidencian el efecto fitoestimulante del FitoMas-E sobre el crecimiento y el desarrollo de posturas de cafeto. Se determinó que la aplicación de 1 L ha^{-1} FitoMas-E es la más adecuada para las variables de crecimiento evaluadas (biomasa seca y área foliar). En el momento de la fase final del vivero, el mejor comportamiento se obtuvo en las plantas a las que se les aplicó el tratamiento de biofertilización de micorriza y FitoMas-E, combinados durante todo el experimento, en el sustrato de pulpa de café. Esta combinación logra reducir hasta un 25 % el fertilizante mineral con resultados superiores a la aplicación del 100 % del mismo.

Palabras clave: cafeto, vivero, micorriza, FitoMas-E, fertilizante

[1] Universidad de Guantánamo, Facultad Agroforestal. avenida Che Guevara, km 2 ½, carretera Baracoa, Guantánamo, Cuba.
[2] Centro Universitario Municipal San Antonio del Sur, Guantánamo, Cuba.
[3] Instituto Nacional Ciencias Agrícolas (INCA), gaveta postal 1, San José de las Lajas, Mayabeque, Cuba, CP 32700.
✉ luperio@fam.cug.co.cu

INTRODUCTION

Coffee trees (*Coffea arabica* L.) is one of the crops with economic importance, its world production is high since it is a highly demanded product, hence it constitutes an employment and foreign currency source for several countries of Africa, Asia and Latin America[A].

The Managerial Group of Mountain Agriculture program to increase coffee production looks forward to plant 81,1 million of coffee seedlings by 2010-1015, out of which, 10,1 million are of the canephora specie which accounts for 30 % (6088,7 ha) of the areas to be planted in the whole country[B] and 20 % of the total Cuba's coffee growing areas are of this specie (1, 2).

As to seedling production is concerned, nurseries play a main role in the production of quality plants. It leads to the need of knowing and using nutritional alternatives to optimize the production of different species in nurseries, grow better fed plants and reach 100 % of survival in the studied areas. In that way, it will be possible to reduce the exhaustion of non-renewable resources as soil (3).

Due to the increased price of chemical fertilizers, the scarce reserves of some natural nutrients, and the high energy consumption to manufacture fertilizers, the use of biological alternatives are a must not only as a need of agricultural production, but also in the future scientific agriculture without affecting ecology and following an economic feasibility.

In this order, it is guaranteed that the application of biofertilizing microorganisms are a viable choice to plant nutrition since they have a triple effect on the soil and the plant's rhizosphere, making possible the assimilation of nutrients and depositing phytohormonal exudates that stimulate plant growth and act as antagonists of pathogen fungi[C].

It is necessary to point out that biofertilization is an alternative to agriculture, its value lies on achieving an efficient inoculation of AMF fungi and the correct application of FitoMas-E, because, when this is achieved, the capability of crops to uptake nutrients and water increases as well as the protection against pathogens.

Thus, the association of microorganisms of different microbiological nature is a favorable practice for crops development, reduction of the vegetative cycle and fertilizers consumption (4). Likewise, it has been demonstrated that FitoMas-E application can reduce the NPK rate in rice (*Oryza sativa*, L.) up to 50 % (5).

Taking the above-mentioned elements into consideration, the present study was conducted to evaluate the use of FitoMas-E andy EcoMic® to reduce the use of mineral fertilizers in coffee seedling propagation to produce coffee seedlings with adequate agricultural production.

MATERIALS AND METHODS

The research was conducted at the nursery of the Basic Enterprise Unit of Puriales, San Antonio del Sur municipality, Guantánamo province, at 300 m a. s. l. in three periods: April-September 2012 (experiment 1); October 2012-March 2013, (experiment 2); April-September 2013 (experiments 3), where reduced fertilizer rates were used with biological alternatives in seedling production. The coffee variety used was Isla 6-14 and seedling production was done in poly bags of 14 x 20 cm.

Beds of 1,20 m width x 20 m long with 144 seedlings were made to make up the treatment in each case. A sample of 10 plants per treatment was taken for evaluations.

In all cases, seeds from the Municipal Agricultural and Livestock Enterprise of San Antonio del Sur were used, at the rate of two seeds per bag of the variety Isla 6-14, sown on a substrate prepared with loose Syalitic Brown soil without carbonates (6) and cow manure (trials 1 and 2 and coffee pulp in the second and third trial) at the rate of 3:1 v/v. Nitrogen content was 1,15 %; phosphorus 0,17 % and potassium 0,50 % for cow manure, while for the coffee pulp it was 3,25 % of nitrogen; 0,39 % phosphorus and 1,69 % of potassium.

Figure 1 shows the climatic data of the location where the trials were done, it also shows the mean values for this location from January 2012 to December 2013 which coincides with the evaluation period. It can be seen that in general variables like temperature, rainfall and relative humidity from May to October, increased their monthly averages which is typical in the rainfall pattern of this area.

[A] Joao, J.P. *Efectividad de la inoculación de cepas de HMA en la producción de posturas de cafeto sobre suelos Ferralítico Rojo Compactado y Ferralítico Rojo Lixiviado de montaña* [Tesis de Maestría], Instituto Nacional de Ciencias Agrícolas, La Habana, Cuba, 2002.

[B] Sánchez, C. *Manejo de las asociaciones micorrizicas arbusculares en la producción de posturas de cafetos (C. arabica* L.*) en algunos suelos del Escambray* [Tesis de Doctorado], Instituto Nacional de Ciencias Agrícolas, La Habana, Cuba, 2001, 103 p.

[C] Mengana, A. *Manejo del suministro hídrico en el cultivo de la cebolla (Allium cepa) con empleo de alternativas biológicas en el Municipio El Salvador* [Tesis de Maestría], Guantánamo, Cuba, 2011, 61 p.

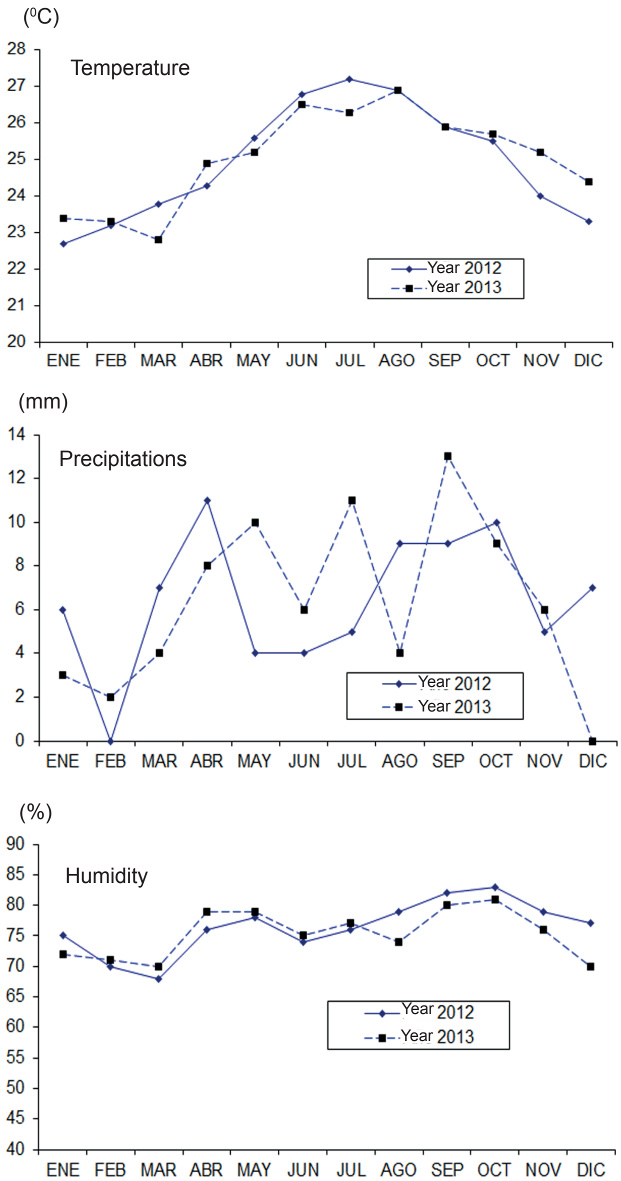


Figure 1. Climatic variables evaluated during the trial

The studied treatments per trial were:

Trial 1

T1 – Absolute control (without application)
T2 – 0,5 L·ha^{-1} FitoMas-E
T3 – 1,0 L·ha^{-1} FitoMas-E
T4 – 1,5 L·ha^{-1} FitoMas-E
T5 – 2,0 L·ha^{-1} FitoMas-E

Trial 2

Cow manure:
T1 - S. E. Cow (Control)
T2 – AMF 1%
T3 - FitoMas-E (1L·ha^{-1})
T4 - AMF 1%+ FitoMas-E (1 L·ha^{-1})

Coffee pulp:
T5 - S. P. Coffee (Control)
T6 – AMF 1%
T7 - FitoMas-E (1L·ha^{-1})
T8 – AMF 1% + FitoMas-E (1L·ha^{-1})

Trial 3

T1 – Absolute control (without application of fertilizers and no alternatives)
T2 – 100 % NPK (4 g)
T3 – 75 % NPK (3 g) + AMF 1%+ FitoMas-E (L·ha^{-1})
T4 – 50 % NPK (2 g) + AMF 1% + FitoMas-E (L·ha^{-1} ^{1})
T5 – 25 % NPK (1 g) + AMF 1%+ FitoMas-E (L·ha^{-1})
T6 – 0 % NPK (0 g) + AMF 1%+ FitoMas-E (L·ha^{-1})

When the mycorrhizal isolate *Glomus intraradices* was used by pelletizing seeds at the rate of 1 % of their weight at the sowing time, FitoMas-E was applied at 1 L ha^{-1} in the second and third trial.

In order to characterize plant growth and mycorrhizal efficiency, different variables were evaluated by taking 10 plants per treatment at transplanting seedlings.

Evaluated variables

- **Foliar Area (FA); (cm^2):** this variable was estimated from the linear dimensions of the leaves and according to the following formula: AF =length x width x 0.64 (7).
- **Total dry biomass (Bs); (g): (Foliar dry mass, Root dry mass, Total dry mass):** plants were cut into sections (leaves-stems, root and total). Roots were carefully washed to remove all the substrate from the bag, then all sectioned plants were introduced into an oven at 65 ^{0}C for drying, till reaching a constant mass. This evaluation was done at the end of the nursery stage.
- **Efficiency Index (EI); (%):** it was used to determine the effect of the mycorrhizal inoculation from the formula proposed by Sánchez (2001)[B]. In this case, such formula was applied to foliar area and dry biomass variables taking non-inoculated plants as control with the same fertility level and the same soil-organic matter relationship.

The formula used was the following:

$$EI(\%) = \frac{F.A\ .Inoculated\text{-}F.A.Control}{F.A.\ Control}$$

Statistical analysis

In all cases, experimental results were subjected to simple ANOVA for experiments 1 and 3 and double ANOVA for experiment 2), applying the Duncan's multiple range test with P≤0,05 % as the comparative criterion among the treatments. All statistical analyses were processed with the statistical package STATISTICA (8).

RESULTS AND DISCUSSION

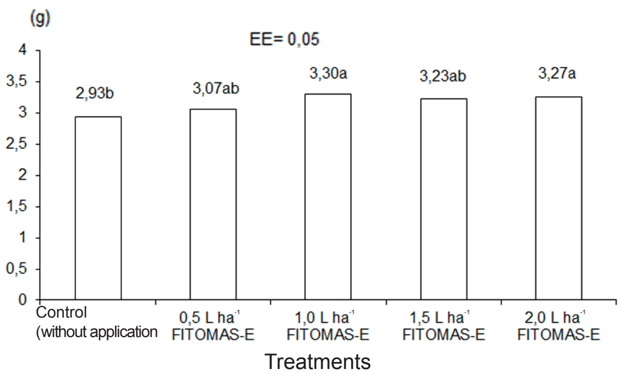
The discussion and interpretation of the results will be done according to the total dry biomass using the criteria provided by authors[A, B] that have worked with this crop:

Experiment 1: evaluation of coffee seedling response to different rates of FitoMas-E.

Growth is an irreversible increase of the plant size generally associated to an increased dry mass[D] and indicates quantitative changes taking place during development. Figure 2 shows the growth evaluation as to total dry biomass in coffee plants treated with different rates of FitoMas-E and in general, the best results were seen when plants were treated with the plant stimulant from 0,5 L•ha^{-1} to 2 L•ha^{-1}.

At 180 days (final nursery stage) this variable reached a magnitude of 2,93 g average per plant in the treatment without FitoMas-E (Control) with significant differences compared to the treatments where the plant stimulant was applied. In these treatments, a difference with values ranging from 0,13 g to 0,37 g increase in plants treated with different rates of FitoMas-E was found. Such differences account for 4,55 % to 12,50 %, a value that belongs to the variant of applying 1 L ha^{-1} of this product.

It is worthy to stand out that whenever FitoMas-E was applied, good results were found in this variable, from the rate of 0,5 L•ha^{-1} to 2 L•ha^{-1}. However, there were no significant differentes among these variants.

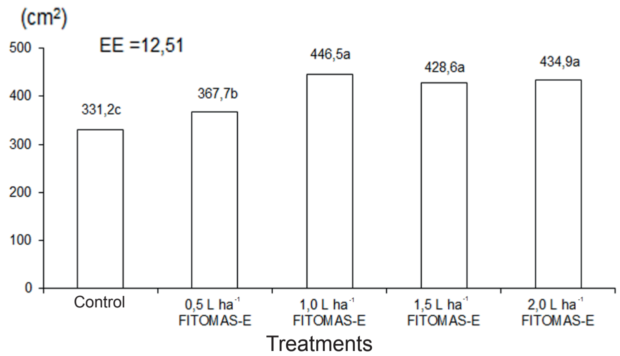


Medias con letras diferentes, difieren significativamente para P≤0.5%, según dócima de Duncan

Figure 2. Total dry biomass of coffee plants treated with different doses of PhytoMas-E

The results of this research prove the phytostimulant effect of FitoMas-E on the growth and development of coffee seedlings, which confirms other studies (9, 10). These authors coincide that this product has significant physiological and metabolic effects on the biological cycles of the crops. FitoMas-E is able to stimulate cell division and enlargement as well as crop nutrition which in turn favors plant growth and development, in addition to germination (11).

The results as to foliar area showed a similar behavior to that of the total dry mass (Figure 3). The best behavior was reached with the variants that received FitoMas-E from 1 L•ha^{-1} to 2 L•ha^{-1} at the end of the nursery stage.



Means with different letters are significantly different according to Duncan's P≤0.5 %

Figure 3. Foliar area of coffee seedlings treated with different rates of FitoMas-E

In this variable the treatment that did not have the biological alternative received 0,5 L•ha^{-1} and showed the lowest values. The reductions seen in these treatments were mainly due to the decreased number and size of the leaves (data not shown), caused by the low nutritional level of the substrate for not having FitoMas-E in one case and a low rate on the other hand during the biological cycle of the crop.

At the end of the nursery stage, this variable reached approximately 447 cm^2 average per plant in the treatment that received FitoMas-E at a rate of 1 L•ha^{-1} without significant differences with the treatments where the phytostimulant was applied at 1,5 L•ha^{-1} and 2 L•ha^{-1}. These treatments showed differences regarding the control with values from 37 cm^2 to 115 cm^2, such differences account for 11,03 % to 34,82 %.

[D]Barroso, L. *Crecimiento, desarrollo y relaciones hídricas de la Albahaca Blanca (Ocimun basilicum* L.*) en función del abastecimiento hídrico* [Tesis de Ingeniería], Instituto Nacional de Ciencias Agrícolas, La Habana, Cuba, 2004, 112 p.

The incorporation of the phytostimulant by the leaves to coffee seedlings, probably caused an increased level of minerals and nitrogen on the plants. Nitrogen plays a key role as forerunner of the number of leaves and a greater foliar expansion due to a higher number and size of the cells.

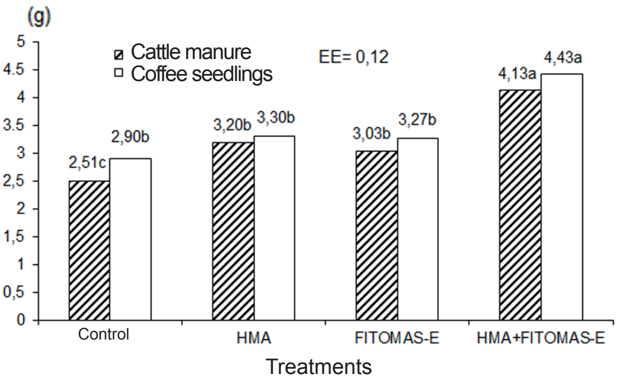
The increased foliar area in treatments with FitoMas-E, is the physiological response of coffee plants. Moreover, it is present in all molecules and proteins being part of the elements involved in the photosynthetic and respiratory activity, so it improves both plant metabolism and its growth making possible for the plant to express its potential to produce more growth.

In all treatments where the phytostimulant was used from 1 L·ha^{-1} there was a favorable effect on the evaluated indicator for coffee seedlings, which could be due to the fact that the product has nitrogen basis, polypeptids, N, P, K, among other chemical properties (12) that contribute to plants growth.

When FitoMas-E was evaluated in the forest specie *Albizzia cubana* under nursery conditions, growth behavior was improved compared to the control (9). Such results match those of this experiment.

Experiment 2: coffee seedling response when mycorrhizae and FitoMas-E are applied in two organic substrates.

From the results of the above-mentioned trial in which the rate of 1 L·ha^{-1} de FitoMas-E was the best rate, this trial evaluated the response of coffee seedlings to the combination of two organic sources, the mycorrhizal biofertilizer and the biostimulant FitoMas-E.



Means with different letters are significantly different according to Duncan's P≤0.5 %

Figure 4. Total dry biomass of coffee seedlings treated with different nutritional alternatives on two organic substrates

The total dry biomass of coffee seedlings treated with different nutritional alternatives and combined with two organic substrates can be seen in Figure 4. In general, there were significant differences among treatments, the best results were attained when mycorrhizal and FitoMas-E were combined regardless the substrate.

The final nursery stage coincides with the evaluation of seedlings, the best behavior was recorded for the treatment where plants were kept under the combined treatment mycorrhizae plus FitoMas-E which showed the effectiveness of applying biological alternatives on a combined basis. Positive results were attained with the use of combined biological alternatives (Pectimorf + Ecomic) that stimulated growth and development for the horticultural specie known as wild radish (13).

This treatment reached a difference of 1,63 g (cow manure substrate) and 1,53 g (coffee pulp substrate) regarding the control, a value accounting for 64,89 % and 52,87 respectively.

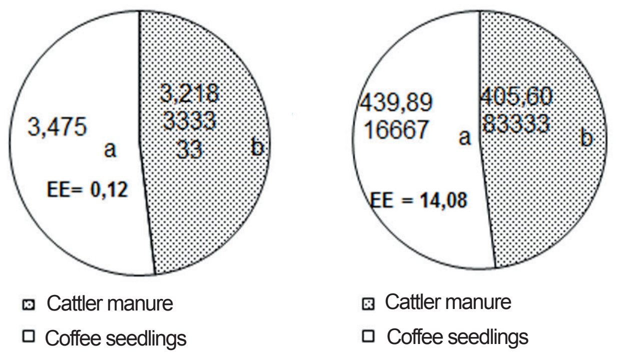
These results show the feasibility of using biofertilizers in the crop, mainly when combined and the substrate is not well represented by a good nutritional reserve. This result coincides with those from other authors (14) who have also reported plants tolerance to a wide spectrum of biotic and abiotic stresses with the use of brassinosteroids.

The technology of beneficial or efficient microorganisms is a viable alternative for every coffee grower it is considered economic, of easy implementation and application, and that agricultural yields prove to lead to superior quality results (15).

An interesting element to stand out is that when both alternatives were independently used, they showed similar results regardless the substrate.

It is evident that as in experiment 1, FitoMas-E was a determining factor in the average dry mass production per plant. However, this indicator favored itself more by combining the phytostimulant with the mycorrhizae, which shows the positive and synergic effect of both products within the plant. These results match those found with the forest specie *Albizzia cubana* under nursery conditions (9).

Figure 5 shows the comparison of both organic sources used to produce coffee seedlings using the studied variables. In both cases, the substrate made up of coffee pulp showed the best results.



Means with different letters are significantly different for P≤0,5 %, according to Duncan's Multiple Range Test

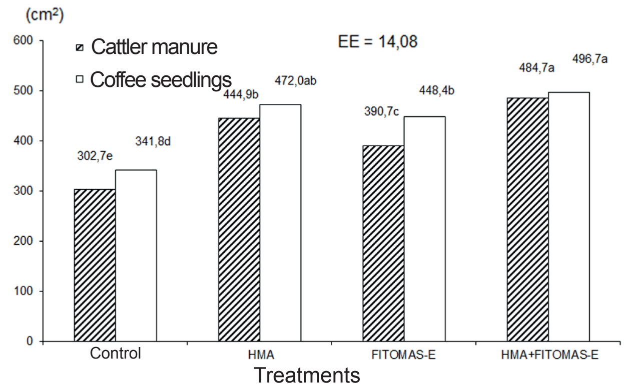
Figure 5. Total dry biomass and coffee seedling foliar area. Seedlings were grown on two organic substrates

It is worth to point out that regardless the evaluated variable, the organic coffee pulp source showed the best results with better means of significant differences compared to the cow manure source; the difference was 0,26 g for the total dry biomass and 31,28 cm^2 for the foliar area variable, values that account for 8,07 % and 8,45 % respectively. These results are influenced by the nitrogen 3,25 %; phosphorus 0,39 % and potassium 1,69 % contents, higher for the coffee pulp than for the cow manure substrate that only showed the following percentages: nitrogen 1,15 %; phosphorus 0,17 % and potassium 0,59 %.

Though it is true there are no differences between both substrates, it is a must to remark that for control seedlings there were indeed differences between them, something that indicates a higher efficiency of the mycorrhizae over the cow manure substrate for the fact of existing a lower difference between control plants and the combined variable.

The difference shown by these two substrates could be due to the fact that plants develop well under optimum nutrients supply, especially of those elements with little mobility like phosphorus, and respond to difusion mechanisms in such a way that there is a net gain for the plant with this association, energy and plant metabolic products that are used for the development of mycorrhizal fungi. They will be largely distributed by the increased uptake and the net growth rate[A].

Figure 6 shows the foliar area of coffee seedlings treated with different nutritional alternatives and organic substrates. It was found that treatments used brought about significant differences in the evaluation done. The best results were reached in treatments where combined biological alternatives were used, without differences between the two organic sources.



Means with different letters are significantly different for P≤0.5 %, according to Duncan's Multiple Range Test

Figure 6. Foliar surface of coffee seedlings treated with different nutritional alternatives on two organic substrates

When both biological alternatives were applied individually, that is, mycorrhizal fungi plus FitoMas-E, the response was different for the cow-manure organic substrate and FitoMas-E. It seems that the phytostimulant is more effective when the substrate is made up of coffee pulp than when it is composed of cow manure.

In the same way previous results have been evaluated, the worst results in the foliar area variable were found when plants were grown under technical standards conditions (3:1 soil: cow manure). However, the combined application of mycorrhizal and FitoMas-E notably favored the production of foliar area by achieving a significant increase of this growth variable compared to control plants, with values from 154,9 cm^2 to 182,0 cm^2 that account for 45,31 % for coffee pulp and 60,12 % for cow manure.

The phytostimulant effect of FitoMas-E has been proven in crops of agro-economic interest like: tomato, pepper, cucumber, string beans, sugarcane, radish, tobacco, water melon, strawberry, among others (10). This beneficial effect is related to the chemical composition of FitoMas-E. In different studies (12) researchers determined the profile of present aminoacids and found that the liquid phase of the product contained 16 aminoacids, a necessary element for the synthesis of protein. Likewise, it has a nitrogen basis since nitrogen is involved in the DNA and in the RNA synthesis (16).

These results coincide with those reached in cocoa when two mycorrhizal isolates were evaluated on a brown soil. The isolates used behaved efficiently and assured a greater foliar area and vigor index for seedlings[E].

[E] Aranda, R. *Diferentes fuentes de materia orgánica y cepas de hongos micorrizógenos en la producción de posturas de cacao* (*Theobroma cacao* Lin.) [Tesis de Maestría], Facultad Agroforestal de Montaña Guantánamo, 2010.

Authors like (3, 17) working with mycorrhized forest species have said that the physiological development of the plant is due to the increased phosphorus uptake through the symbiotic formation of root organs, thus allowing that plants be more resistant to those negative changes that could be present in an ecosystem.

The behavior of the mycorrhizal efficiency index on two organic substrates from the total dry biomass and foliar surface is shown in Figure 7, and in general its percentages ranged from 13,79 % and 46,98 %, with the best results present in the organic substrate of cow manure. It could be caused by the capability of the mycorrhizae to increase efficiency in cases of low fertility.

A better mycorrhizal development was found when there were conditions of lower nutrient availability in substrate[A], in whose conditions, mycorrhizae isolates showed the greatest agrobiological effects on coffee seedling development.

It is important to point out that the inoculation with efficient AMF isolates increased the macroelement contents (N, P and K) in plants, indicating that mycorrhization is not only linked to phosphoric nutrition as different authors state (9, 18), but with other elements, which suggests that once plants are mycorrhized, this mechanism increases uptake of elements like P, N, K, Ca, Mg, Zn, Cu, Mo, B (19).

Total dry biomass showed the lowest percentages, coffee pulp recorded the worst behavior. The percentages of this variable were around 13,79 % to 27,66 %. However, the foliar area variable was the most expressive one of this index.

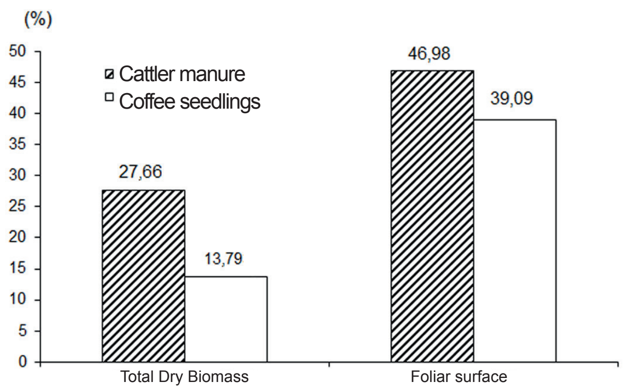
The low mycorrhizal efficiency indexes for the studied variables when coffee pulp was used, can be influenced by the higher nitrogen (3,25 %), phosphorus (0,39 %) and potassium (1,69 %) content compared to cow manure whose values are lower (1,15 %, 0,17 %, 0,50 % in the same order). The fungal hyphae have a greater affinity by the phosphate ion when its concentration in the solution is low (20), hence the high mycorrhizal efficiency index for the cow manure.

In relation to AMF inclusion into a fertilization system with cow manure there are reports saying that the quantity of nutrients to apply to reach a certain yield level are usually lower than the necessary ones to reach that yield without inoculation (21).

However, though the behavior of mycorrhizal efficiency has been better in the substrate containing cow manure, it was not equivalent to a better morphology of coffee seedlings. This phenomenon could be associated to the nutritional richness coffee pulp has that guarantees a higher nutrient availability in the substrate to be uptaken by the plant.

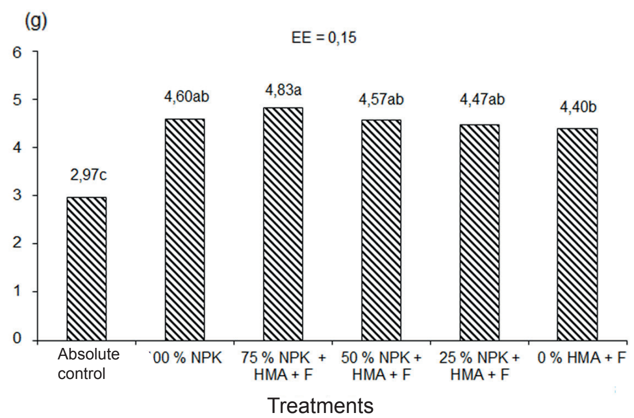
Experiment 3: evaluation of the Micorrhyzae-FitoMas-E combination with reductions of chemical fertilizers.

The total dry biomass of coffee seedlings treated with the combination of FitoMas-E and mycorrhizae and different N-P-K levels is shown in Figure 8. Significant differences were found among treatments; the best results were found when chemical fertilizers were applied.



Means with different letters are significantly different according to Duncan's P≤0,5 %

Figure 7. Behavior of the Mycorrhizal Efficiency Index in two organic substrates from the total dry biomass and foliar area



Means with different letters are significantly different according to Duncan's P≤0.5 %

Figure 8. Dry biomass of coffee seedlings treated with FitoMas-E, Mycorrhizae and different N-P-K levels

An important issue to point out is that when 100 % of mineral fertilization was used, plants did not show differences with those where 75 %, 50 % and 25 % of the same rate was used, provided the presence of the combination of the biofertilizer and the plant stimulant. It could be related to experimental conditions (the combination of AMF and FitoMas-E), that on one hand, mycorrizae increased the uptake efficiency of nutrients and water, and on the other hand, FitoMas guaranteed a quick uptake of nutrients through the stomas making possible for the plant reaching the necessary NPK and develop their optimum growth potential.

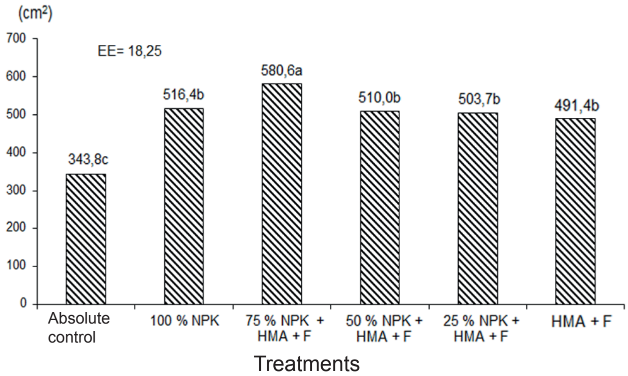
It is also possible that the AMF and FitoMas-E combination has improved the water conditions of the plants due to the benefits provided by mycorrhizal fungi under stress conditions. In this regard, there is significant correlation between the soil water content and the efficiency in the use of nitrogen[F], therefore, an efficient water level guarantees a plant without water stress, which increases the growth rate because of an increased biomass. This aspect is also associated to the phosphorus availability in the soil.

This positive growth effect explains the increased availability of nutrients for the host plant since it favors the AMF action. These studies (22) showed a similar response to increased microbial communities when a substrate made up of soil and filter-press mud was used. It is also stated that the inoculation of efficient AMF isolates combined with the application of mineral or organic fertilizers (23), increases the efficiency of nutrients and reduce the rates of organic or mineral fertilizers.

Previous studies have already reported the direct benefits of combining organic fertilizers and the inoculation of arbuscular mycorrhizae to crops, mainly on plant growth (24, 25), which coincides with the results of these trials.

The foliar area of coffee seedlings treated with the combination of of FitoMas-E and mycorrhizal fungi at different levels of N-P-K is shown in Figure 9. Significant differences were attained among treatments. The best results were for the treatment of 75 % N-P-K and the combination of mycorrhizae and FitoMas-E.

When the combination of biological alternatives was applied together with 75 % of mineral fertilization, the best result in the variable foliar area was reached, with a difference of 237 cm^2 compared to the absolute value of the control that accounts for 68,89 % increase.



Means with different letters, are significantly different for P≤0,5 %, according to Duncan's Multiple Range Test

Figure 9. Foliar area in coffee seedlings treated with FitoMas-E, Micorrhyzae and different levels of N-P-K

Likewise, there was a difference of 65 cm^2 compared to 100 % of N-P-K application which accounts for 12,59 %.

In the treatment where plants grew up only with the rate of 3:1 soil: coffee pulp, lower values for this variable were found due to the absence of chemical fertilizers during the nursery stage and not having the combination of AMF and FitoMas-E that could supply such difference.

Probably, these plants did not have a good water balance and nutrients availability at certain times of the growth cycle because of the absence of the studied combination. It influenced on the reduced foliar area of the plant, mechanism developed by some plant species when exposed to limited moisture levels in order to reduce transpiration losses[E].

In this context, it is stated that plants suffering from phosphorus deficiencies reduce foliar expansion[G], with a lower foliar area and a smaller number of leaves, associated with yellowing and premature senescence of the mature leaves. In contrast, the protein and chlorophyll contents per foliar area unit is not very much affected.

Though the chlorophyll content was not determined, it is frequent, under these conditions, it is higher than in deficient plants which provides leaves with a dark green color, however, photosynthetic efficiency per chlorophyll unit is much lower. Areal growth is more depressed than the root one, providing plants with a higher rate of carbohydrates for the roots.

[F] Gleddie, S.C.; Schlechte, D. y Turnbull, G. "Effect of inoculation with *P. bilaii* on phosphate uptake and yield of canola in Western Canada", *Proceed. Alberta Soil Sciencie Workshop*, Edmonton, Alberta, Canadá, 1993, pp. 155-160.

[G] Pérez, A. *Fertilización y requerimientos de nitrógeno para plantaciones de Coffea canephora Pierre ex Froehner var. Robusta cultivada en suelos Pardos de la región oriental premontañosa de Cuba* [Tesis de Ingeniería], Instituto Nacional de Ciencias Agrícolas, Mayabeque, Cuba, 2011, 98 p.

All this leads to an underuse of ecotystem resources as radiation and water which determines lower green biomass production.

In studies dealing with reduced nitrogen fertilization by using FitoMas-E, in garlic, the best response from the economic point of view was attained with the rate of 1,0 L•ha^{-1} of FitoMas-E + N at 50 %. (26).

It is interesting to stand out that the results from this research help assist the productive reality because is supposes that the combined application of EcoMic® and FitoMas-E makes possible to reduce 25 % of the current chemical fertilizer consumption. It is indicative that with the same quantity of substrate and less quantity of fertilizers, quality seedlings can be produced with a good foliar area as referenced by different authors.

CONCLUSIONS

- ♦ FitoMas-E (1 L•ha^{-1}) application increases growth and development in coffee seedlings which is reflected in the foliar surface as compared to control plants.
- ♦ Out of the organic sources used in the preparation of the substrate, coffee pulp showed the best results with values around 8 % increase compared to cow manure.
- ♦ The combination of mycorrhizae and FitoMas-E reduces 25% of the mineral fertilization used for coffee seedlings production system.

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