Cultivos Tropicales, 2022, vol. 43, no. 2, e03
ISSN impreso: 0258-5936
ISSN digital: 1819-4087

http://ediciones.inca.edu.cu

abril-junio
Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas

Original article

Effect of co-inoculation of efficient microorganisms - AMF on tomato (*Solanum lycopersicum* L.) crop yield

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is the most widely distributed vegetable in the world. In Cuba, it is one of the most productive and can be cultivated in all provinces of the country, its demand increases continuously and with it its cultivation, production and trade. However, an inadequate phytotechnical management of the crop imposes the search for alternatives that contribute to increase its development and yield. The present work was developed with the aim of evaluating the effect of efficient microorganisms (EM)-arbuscular mycorrhizal fungi (AMF) co-inoculation on tomato crop. The experiment was developed in the experimental areas of the National Institute of Agricultural Sciences (INCA) located in San José de las Lajas municipality, Mayabeque province, Cuba. The EM interaction with the certified mycorrhizal inoculant of INCAM-4 strain, *Glomus cubense* species, was evaluated, where four treatments were studied under a randomized block design with four replications. Co-inoculation of AMF and EM stimulated seedling growth at the seedling stage by 18-32 %, compared to the control. Nutritional status of plants was superior, as well as the increase of the fungal variables evaluated in each of phenological stages of the crop. The agricultural yield of the crop doubled due to a higher number of flowers, fruits per plant and percentage of fructification, demonstrating the positive interaction between both bioproducts.

Key words: vegetable, microorganisms, mycorrhiza, yield

INTRODUCTION

The application and use of bioproducts for the benefit of agriculture is expanding strongly in Cuba [(1)]. Several researches obtained and applied have approached the co-inoculation of microbial products to achieve positive

effects on crops and to be part of the alternatives to reduce external inputs and guarantee greater efficiency in the use of mineral fertilizers [2,3].

Among different actions, there is a growing use of biological products such as arbuscular mycorrhizal fungi (AMF), which constitutes an alternative for producers who are committed to agroecological production. In Cuba, several investigations are directed to the benefits that AMF contribute to agricultural productivity, with a large number of studies demonstrating the positive Ecomic® influence for different crops such as grasses, roots, tubers [4,5] and vegetables [6,7].

On the other hand, Efficient Microorganisms (EM), developed since the 70's, show that they reestablish the microbiological balance of the soil, improving its physical-chemical conditions, increasing crop production and its protection, as well as conserving natural resources, generating a more sustainable agriculture and environment [8-10].

Several results point to the effectiveness of mixed inoculations with mycorrhizal inoculants [11] and other biostimulants. However, studies referring to the co-inoculation of AMF and EM are little addressed in tomato cultivation.

Tomato (*Solanum lycopersicum* L.) is the vegetable with the highest national production, with an average yield of 12.02 t ha^{-1}. In most tropical countries, its yield is affected by biotic and abiotic factors that cause a considerable decrease in yields. This situation is compounded by inadequate phytotechnical management of the crop, which makes it necessary to look for alternatives that contribute to increase its development and yield [12,13]. The general aim of this work was to evaluate the effect of the co-inoculation of efficient microorganisms-arbuscular mycorrhizal fungi on tomato crop.

MATERIALS AND METHODS

The study was conducted in the period from October to December 2017, in the areas of the National Institute of Agricultural Sciences (INCA), in a Ferrallitic Red soil, according to the Cuban Soil Classification [14]. The chemical characteristics of this soil are shown in Table 1.

Table 1. Chemical characteristics of the Ferrallitic Red agrogenic leached soil, at 0-20 cm depth

pH (H_2O)	C	OM	K_2O	P_2O_5	Na^+	Ca^{2+}	Mg^{2+}	Ca/M	P
	($g\ kg^{-1}$)		($mg\ kg^{-1}$)		($cmol\ (c)\ g\ kg^{-1}$)				(ppm)
7.5	23.0	39.8	201.1	780.8	0.13	16	2,6	6	341

To evaluate the co-inoculation effect of efficient microorganisms (EM-Lebame®) with Arbuscular Mycorrhizal Fungi (AMF-Ecomic®), the cultivar "Mara", from INCA's Plant Breeding Program, was selected. Four treatments were studied (Table 2), distributed in a completely randomized design with three replications, to which mineral fertilization was applied at a dose of 1 t ha^{-1} of NPK [9-13-17].

Table 2. Treatment studied

	Treatments	
	Seedbed	**Trasplant**
T1	AMF	AMF
T2	AMF +EM	AMF +EM
T3	EM	EM
T4	Control (without AMF and EM)	Control (without AMF and EM)

The experimental area of each seedbed treatment was 2 m^2, to which organic fertilizer was applied at the rate of 1 kg m^2 of cow dung. The AMF were inoculated by seed coating technology, using the certified inoculum of INCAM-4 strain, *Glomus cubense* species [15], which contained 37 spores g^{-1}. This strain is reproduced in the mycorrhizal strain of the Department of Biofertilizers and Plant Nutrition from INCA.

The EM were applied in the form of the commercial product Lebame®, obtained by the Cuban Research Institute of Sugar Cane Derivatives (ICIDCA), composed of a combination of microorganisms of the genera *Bacillus subtilis* B/23-45-10 Nato, *Lactobacillus bulgaricum* B/103-4-1 and *Saccharomyces cervisiae* L/-25-7-12, with a titer of 10^6 UFC mL^{-1} [16]. The dose of 1.5 L ha^{-1} was applied as a foliar spray at 10 days after sowing (DAS).

At the transplanting stage, the experimental plots had a total area of 15 m^2, in a planting frame of 1.20 m x 0.30 m. The experimental plots were composed of four rows of four rows of four rows. These plots were composed of four 3 m long furrows where 40 tomato plants were transplanted per plot at 25 DAS. In this phase of the experiment, only the EM were inoculated seven days after transplanting. The application was carried out in the same way as described after planting. A randomized block design with four treatments and three replicates was followed. Cultural attentions were carried out as recommended by the Technical Manual of the crop [13].

The evaluations carried out on 15 plants per treatment, taken at random 20 days after germination, as well as those carried out during the flowering-fruiting stage and harvest, are shown below.

- Height (cm): with a graduated ruler, it was measured from the root collar to the axil of the youngest leaf.
- Root length (cm): with a graduated ruler, the main root of the crop was measured.
- Number of leaves: visual count.
- Leaf dry mass (g): by weighing on an analytical balance with an accuracy of ± 0.01 mg and drying in an oven at 70 °C, until constant mass.
- No. of flowers $plant^{-1}$: visual count.
- No. of fruits $plant^{-1}$: visual count.
- Fruiting %: result of dividing the number of fruits/plant by the number of flowers/plant, expressed as a percentage.

- Foliar NPK content (%): by acid digestion (wet ashing) with H_2SO_4 + Se, according to Kjeldahl method and calorimetric determination with Nessler reagent and molybdenum blue for N and P, respectively, and flame photometry for K [17]. Samples were taken at the flowering stage, between the third and fifth pair of leaves (15 leaves per sample), adjacent to flowering.
- Average fruit mass (g): result of dividing the total fruit mass by the number of fruits in the plot.
- Agricultural yield/surface area (t ha^{-1}).

In addition, samples were analyzed in the mycorrhizal laboratory of INCA. For the estimation of indicators of colonization frequency and visual index, rootlets were stained using the Parker Dye technique [18]. Subsequently, the following fungal variables were evaluated:

- Frequency of radical colonization: according to the intercept method [19].
- Visual index: by Trouvelot method [20].

The statistical processing of the experimental data was carried out through an Analysis of Variance (ANOVA) of simple classification for seedbed data and of double classification for the data obtained at the transplanting stage. Percentage (%) mycorrhizal colonization and fruiting data were transformed by the function arcsen√x. In cases where significant differences between means were found, they were compared by Duncan's tuple for 5 % significance. The analyses were performed with the Statgraphics Centurion program (version 15.1).

RESULTS AND DISCUSSION

Effect of EM and its interaction with AMF on plant growth variables

A positive effect was observed in all inoculated treatments (Table 3).

Table 3. Effect of AMF-EM on the growth of tomato plants (var. Mara) at 30 DAG

Treatments	Foliar dry mass (g)	No. of leaves	Root length (cm)	Height (cm)
T1. AMF	1,74 a	6,6 b	13,6 b	17,1 b
T2. AMF+EM	1,80 a	6,8 a	17,6 a	21,8 a
T3. EM	1,73 a	8,2 a	17,3 a	22,4 a
T4. Control (without AMF and EM)	1,47 b	6,2 b	13,1 b	17,2 b
SE x	0,03*	0,36*	0,3*	0,39*

Means with common letters do not differ significantly according to Duncan's test ($p \leq 0.05$).

Plants inoculated with AMF and the control showed statistically similar results for most of the variables studied. They showed an increase in leaf dry mass of 19 % with respect to the control, which could be related to the increase in other indicators not evaluated, such as stem diameter and leaf size.

Several studies report that mycorrhization stimulates the growth of dry biomass. They also point out that AMF-plant symbiosis is typically mutualistic, the fungus depends on the plant to obtain photoassimilates and plant receives numerous benefits in return that allow it to increase its biological yield [4]. In this sense, it is also suggested that the increase of biomass production by increasing the foliar area is important to characterize its productivity, since it reflects the yield of the plant and can generate increases in production [21,22]. Studies

carried out in this crop, showed that the symbiotic association of mycorrhizal fungi in roots of plants, produce diverse changes and modifications at physiological level, among which stand out the increases in photosynthetic activity, due to the effect of the greater capacity of CO_2 fixation and, consequently, the increase of growth rates and biomass produced [15].

EM single inoculation effect of reached values for dry mass, number of leaves, root length and plant height that exceeded the control treatment by 18, 32, 32, 32 and 31 %, respectively. Similar results were obtained when evaluating at 20 DAG, the effect of different dilutions, moments and form of EM (Lebame®) application on this cultivar. The variant of inoculation by foliar spraying of 5 mL L^{-1} 10 DAG reported increases in relation to the control for the same variables of 48; 31.2; 33.9 and 24.5 %, respectively, according to the order mentioned above [23].

The literature also shows other works that evidenced significant results when evaluating the effect of EM on other crops of economic interest, although with higher application rates. In this study, 8, 10, 12 mL L^{-1} of EM (Lebame®) were foliar applied to the sugarcane cultivar C87-51 in the *ex vitro* acclimatization phase 7, 14 and 21 days after transplanting. The same showed no statistical differences between dilutions studied; with 8 mL L^{-1} they reached quality parameters superior to the control and acclimatization time was reduced [16].

Co-inoculated plants showed a similar behavior to those inoculated only with ME; they did not differ statistically. Compared to those inoculated only with AMF, they were statistically superior for most variables, except for leaf dry mass content. This treatment also showed superior results to the control, by 22, 10, 35 and 27 %, respectively, according to the order in which they appear in Table 3.

Other authors have also evaluated the performance of the single and combined application of EM (Lebame®) + AMF (*Glomus cubense*) on (*Capsicum annuum* L). They used the same inoculation forms for both products, although they applied doses of 2.5 L ha^{-1} (10 mL L^{-1}) of EM. In this case, it is important to highlight that the treatments showed similar behavior for the plant height variable [16,24].

Inoculated treatments showed a positive behavior in relation to the control. According to the technical instructions for the crop, they reached the required height (10-18 cm) for transplanting before 30 days. Therefore, from the productive point of view, not only were the growth indicators positively stimulated, but it was also possible to reduce the number of days that the plants were established in the seedbed.

EM effect and its interaction with HMA on agricultural yield and its components

The increase in flowering and fruiting manifested by plants inoculated with AMF and EM showed a statistically superior performance to the control for most of the variables evaluated (Table 4).

Table 4. Effect of AMF and EM co-inoculation on flowering, fruiting and yield of tomato crop (*var. Mara*)

Treatments	Plant			Plot	
	Total fruits	Total flowers	Fructing %	Average mass (g)	Yield (t ha^{-1})
T1. AMF	11,8 c	14,73 b	82,83 b	51,05 b	20,06 c
T2. AMF +EM	13,8 a	16,16 a	85,37 a	55,86 a	27,65 a
T3. EM	12,6 b	15,16 b	83,19 b	51,91 b	21,75 b
T4. Control (without AMF and EM)	9,3 d	11,43 c	80,26 c	41,53 c	12,87 d
SE x	0,1*	0,19*	0,92*	1,5*	0,62*

Means with common letters do not differ significantly according to Duncan's test ($p \leq 0.05$)

The EM (Lebame), according to studies reported by ICIDCA is able to produce between 14 and 18 % of indolacetic acid (AIA) [16], which could stimulate the growth of the crop, from the different physiological mechanisms that stimulate the production of flowers. This results in an increase in the number of fruit set, especially due to the presence of gibberellins and some phytohormones capable of influencing the physiological phases of flowering and fructification [25].

The result shown by some of these indicators allowed corroborating the results reached by other authors who used efficient microorganisms in the cultivation of tomato and other vegetables, managing to stimulate the growth and yield of plants, with significant statistical differences in relation to control treatments [26-29].

When evaluating the agro-productive behavior of the *Zea mays* crop inoculated with EM, it reported an increase with respect to the control of some yield components such as the number of cobs per plant, mass of cobs with straw and mass of cob without straw in 15.8, 14.9 and 29.8 %, respectively [30].

Published studies in horticultural crops demonstrated the positive effect of foliar application of 10 mL L^{-1} of ME when evaluating some yield components in *Brassica oleracea*, *Lactuca sativa* and *Beta vulgaris*, with respect to the control without bioproduct [24]. The yield of *Phaseolus vulgaris* increased statistically in relation to the control when the morphophysiological and productive indicators of the crop were stimulated with the application of EM and different biostimulants [31,32].

On the other hand, arbuscular mycorrhizal fungi constitute an alternative way for the nutrition of plants, by increasing their growth, development and positive effects on yields, especially of tomato [4,33]. Several studies have proven the direct relationship between AMF the presence in the rhizosphere and crop yields. In addition to their effectiveness, efficiency with other microorganisms and compatibility with other bioproducts [34,35].

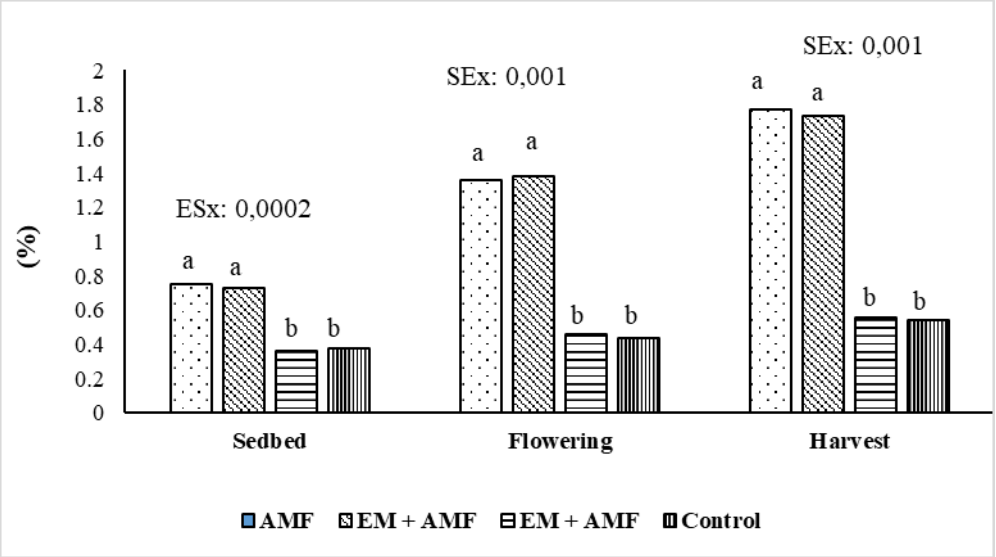
The results of AMF and EM co-inoculation showed a positive interaction between both products, with values exceeding single inoculations and the control treatment in terms of crop yield and its components. This result also significantly exceeds the national average (12.02 t ha^{-1}) achieved by the crop, according to the National Statistics Office [12].

Other authors reported that the application of 10 mL L^{-1} of EM at 10 and 20 days after germination (DAG), caused a stimulus in the production of (*Capsicum annuum* L.) co-inoculated with AMF, from a higher production of flowers, fruits and weight per plant. Similar results were obtained in this experiment where

single inoculations of both products (AMF and EM) and co-inoculation (AMF+IM) significantly outperformed the control without bioproducts [16,24].

Frequency of colonization and visual index in tomato roots

Figures 1 and 2 present the simple analysis of variance of fungal behavior, percentages of colonization frequency (Figure 1) and visual index (Figure 2) evaluated at the end of the seedling stage, at flowering and at harvest.

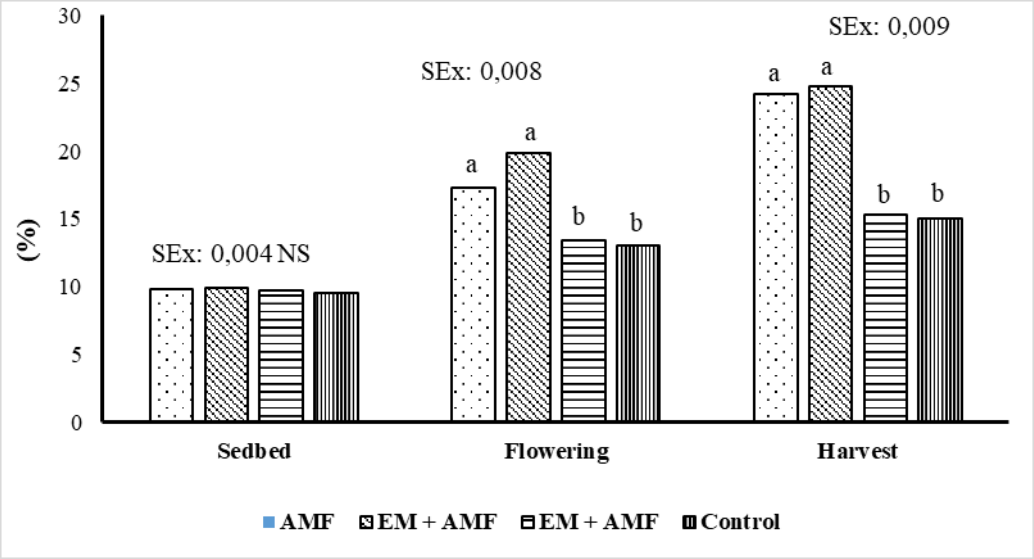


Means with common letters do not differ significantly according to Duncan's test ($p \leq 0.05$).

Figure 1. Behavior of the mycorrhizal colonization frequency at the end of the seedbed, at the flowering stage and at the time of harvest

The presence of fungal structures was detected without statistical differences between the treatments inoculated with AMF at the beginning (30 DAS). These increased in each of the phenological phases of the crop, until significant differences were obtained in favor of the AMF and AMF+EM treatments, which surpassed the EM treatment and the control.

In the co-inoculated treatment, statistically higher colonization frequency values were reached from the second phenological phase (flowering-fruiting) studied (Figure 1), with a difference with respect to the control without inoculation of 7 and 8.33 %, respectively. Similarly, the variant inoculated only with AMF obtained superior results with respect to the control, for the aforementioned variables by 4 and 7.3 %, respectively.



Means with common letters do not differ significantly according to Duncan's test ($p \leq 0.05$).

Figure 2. Behavior of the visual index at the end of the seedbed, at the flowering stage and at the time of harvest

The visual index in the AMF-EM co-inoculated treatment outperformed the control at the three time points evaluated (Figure 2) by 0.49, 1 and 1.26 %, respectively. The AMF-only treatment outperformed the uninoculated control and the EM treatment by 0.49, 1 and 1.21 %, respectively.

Although the percentage of colonization and the visual index of AMF-inoculated variants was significantly higher, mycorrhizal activity was evident in treatments where AMF was not inoculated. This effect could be related to the presence of arbuscular mycorrhizal fungi resident in the soil. This situation is commonly found in experiments under field conditions, where the substrate is not previously sterilized. Some authors found positive effects with the use of a concentrate of native strains (CNS); however, the behavior is sometimes inferior to those inoculated with effective strains [(36)]. Also, the permanence effect of the inoculant of previous cultures in field conditions has been studied [(4)].

The values of root colonization frequency and visual density achieved may have been related to the high

The high values of organic matter, phosphorus and potassium (Table 1) present in the soil where the experiment was carried out may have been related to the high values of organic matter, phosphorus and potassium (Table 1) present in the soil where the experiment was carried out. In addition, the application of mineral fertilizers at the time of transplanting provided an adequate availability of nutrients that reduced the development of mycorrhizal structures, causing a malfunction or even inhibition of the symbiosis depending on the dose applied [(37)].

Taking into account the aspects previously mentioned, low values of frequency of radical colonization and visual density could not be fully expressed; however, the variables studied in each of phenological stages of the crop, such as plant growth and yield corroborate that, even when mycorrhization was not expressed in all its magnitude, both simple inoculation and co-inoculation with EM, surpassed the control without inoculation and EM, evidencing that there was no inhibition of the mycorrhizal symbiosis.

Evaluation of the nutritional status of tomato plants

Leaves are plant tissues that are most responsive to external and internal changes in nutrient supply, as they play a significant role in plant physiology, particularly in the process of photosynthesis and the synthesis of other organic compounds. Therefore, it is considered to be the plant organ that from a metabolic point of view best reflects the nutritional status. For this reason, when evaluating the nutritional condition of a crop, it is commonly done from the concentrations of nutrients in its foliar tissues [38].

N, P and K accumulation was higher in inoculated plants. They showed the highest values and significant increases with respect to the control (Table 5). These results are considered within the adequate range of nutrients for tomato cultivation [39] and show that all inoculated treatments completed their biological cycle without nutritional deficiencies.

Table 5. Effectiveness of AMF-EM co-inoculation on the nutritional status of tomato plants (*var. Mara*) at the flowering-fruiting stage (75 DAS)

Treatments	N	P ($g\ kg^{-1}$)	K
T1. AMF	41,6 c	4,4 c	32,2c
T2. AMF +EM	46,1 a	5,6 a	35,5 a
T3. EM	43,1 b	4,8 b	33,5 b
T4. Control (without AMF and ME)	40,0 d	4,1 d	30,7 d
SE x	0,024*	0,06*	0,22*
CV	5,5	12,39	5,6

Means with common letters do not differ significantly according to Duncan's test ($p \leq 0.05$).

The interaction of AMF+EM reached higher values with marked statistical differences over the rest of treatments. This nutritional state of the plants could have been positively influenced by the additive effect of both inocula, expressing its benefits in the increase of growth indicators evaluated, which subsequently resulted in a greater number of flowers and fruits, causing in turn, an increase in agricultural yields.

The treatment inoculated with EM was statistically superior to that inoculated with AMF and the control. These plants were transplanted with a greater development of their root system and the number of leaves, which could have positively influenced their capacity to absorb nutrients and increase their biological yield at this phenological stage.

Treatments reached N concentrations between 40 and 46.1 g kg^{-1}; adequate values, which are above the deficiency range. These values may be associated with the increase manifested by biofertilization in the formation of vegetative organs and the increase in fruit weight. It is important that the N content be adequate to avoid an imbalance of K and P, which results in excessive vegetative development to the detriment of fruit set; with the production of hollow and light fruits, with little juice, few seeds, succulent stems and overgrown leaves [39].

Adequate P contents are also shown in the plant tissue of all treatments, evidencing values ranging from 4.1 to 5.6 g kg^{-1}. This adequate phosphorus availability accelerates plant root development, improves flowering, fruiting and fruit development. Likewise, the percentages of K, which vary between 30.7 and 35.5 g kg^{-1} may have played an important role in the amount of sugars accumulated by the fruit. Like phosphorus, K helps to increase the amount of dry matter and vitamin C. This macroelement is necessary in tomato for the formation of stems and fruits, synthesis of carbohydrates, increase of solid substances, coloration and fruit brightness [40].

CONCLUSIONS

The results demonstrate the positive interaction of co-inoculation between arbuscular mycorrhizal fungi and efficient microorganisms, with a more accentuated action in the flowering-fruiting and crop yield phases, given by an increase in fungal indicators, reflecting, in general, a better nutritional state of plants.

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