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Original article

Effect of a microbial inoculum on the growth of tomato plants (*Solanum lycopersicum* L.)

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ABSTRACT

Sowing the tomato crop through inoculated seedlings constitutes an ecologically acceptable alternative that guarantees greater growth and reduces external inputs. In order to increase the growth and development of tomato, a study was carried out in a traditional seedbed with the objective of evaluating the most effective dilutions in the growth of tomato plants previously embedded in the seeds in the microbial inoculum (MI). This experiment was conducted under a Completely Random Design with five treatments and five repetitions. The data were through the Statgraphics Centurión program (version 15.1) processed. The imbibition of tomato seeds for 15 minutes in 5, 10 and 15 mL L^{-1} of the MI did not cause statistical differences between them. There is a similarity expressed in the different moments evaluated and for all the variables under study stimulating the height of the plant, the root length, the number of leaves, the diameter of the stem and the foliar and root mass. Taking into account the results obtained in this research, the effect of dilutions on the growth and development of tomato plants was demonstrated. However, even when the three dilutions reached the highest values, to produce plants in less time and with the necessary quality; the imbibition in 5 and 10 mL L^{-1} constituted an alternative that represents a saving for bioproduct to be used.

Key words: efficient microorganisms, biostimulant, seedbed, vegetable

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the most widely spread vegetable in the world and the one with the greatest economic value. It is the second most important species within the genus *Solanum* spp., Due to its role in the eating habits of a large part of the world population, its demand increases continuously and with it its cultivation, production and trade [1,2].

In Cuba, in 2016 the area planted with this crop occupied 40,049 hectares, which represents 21.5 % of the area used for planting vegetables, with a production level of 481 470 tons and an average yield of 12.02 t ha^{-1} [3].

For tomato cultivation, sowing through seedlings ensures greater survival than if done directly from seed, guaranteeing higher yields. Success depends, largely, on the care given to the seedbeds, which allows the obtaining of uniform plants, in a good state of development and without phytosanitary problems, factors that influence greater resistance to the rigor of transplantation and greater percentage of survival in the field [4].

Tomato production in Cuba still does not reach the potential yield, due among some causes to the inadequate phytotechnical management of the crop, which makes it necessary to look for alternatives that can contribute to the increase and development of the same. In this sense the Cuban Research Institute of the Derivatives of Sugar Cane (ICIDCA), developed a microbial inoculum (MI), based on Efficient Microorganisms (ME) technology.

Microbial communities in soils are considered vital to ensure the sustainability of ecosystems. They are responsible for functions such as carbon transformations, nutrient recycling, and maintenance of soil structure and regulation of biological populations [5].

The use of beneficial microorganisms is a viable alternative for productions since they constitute an economically attractive and ecologically acceptable medium. They reduce external inputs, improve the quantity and quality of internal resources, as well as guarantee greater efficiency in the use of mineral fertilizers [6].

At this time, the production and use of bioproducts for the benefit of agriculture is expanding through the integrated work of the Business Group of Pharmaceutical Laboratories (LABIOFAM) and various research centers in the country. Among those that stand out Azofert®, Nitrofix, Fosforina, Dimargon and EcoMic® that can also be used in traditional seedbeds or in root balls or trays.

The MI is being in crops of economic interest such as vegetables studied, sunflower, beans, banana and sugar cane where it has been shown that with foliar applications of doses between 1.5 L ha^{-1} and 3 L ha^{-1} are obtained significant increases in yields [7,8]. However, the studies related to the inoculation of this product in tomato cultivation under seedling conditions have been little addressed, hence the need for this research. Taking into account these criteria, the following scientific problem was raised: How to increase the growth of the tomato crop in the seedbed stage? According to these antecedents, the present work aimed to evaluate the biological effect of a microbial inoculum on the growth of tomato plants in the seedling stage of the crop.

MATERIALS AND METHODS

The experimental work was carried out in the areas of the National Institute of Agricultural Sciences (INCA), located in Tapaste, San José de las Lajas, Mayabeque province. In the experimental farm, "Las Papas" from the Department of Agricultural Services (DSA).

The experiments were developed on a Dystric Agrogenic Leaching Red Ferralitic soil, according to the Classification of the Soils of Cuba, which is correlated with the World Reference Base (WRB) as Nitisol ferralic, lixic, (dystric, rhodic, clay) [(9,10)].

The cultivar studied was tomato (*Solanum lycopersicun* L.), cultivar 'Mara'. An (MI) was studied, obtained by the Cuban Institute for Research on Sugar Cane Derivatives (ICIDCA), composed of a combination of microorganisms belonging to its collection, 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 CFU mL^{-1}, which is marketed under the brand LEBAME® [(7,11)].

A study was in a traditional seedbed carried out with the objective of evaluating the most effective dilutions in the growth of tomato plants previously embedded in the seeds in the IM. They were taking into account defined how many previous studies under laboratory conditions [(12)]. The beds measured 30 m long x 1 m wide to which organic fertilizer was applied at a rate of 1 kg m^2 of bovine manure. This experiment was conducted under a Completely Random Design with 5 treatments and 10 repetitions as shown below:

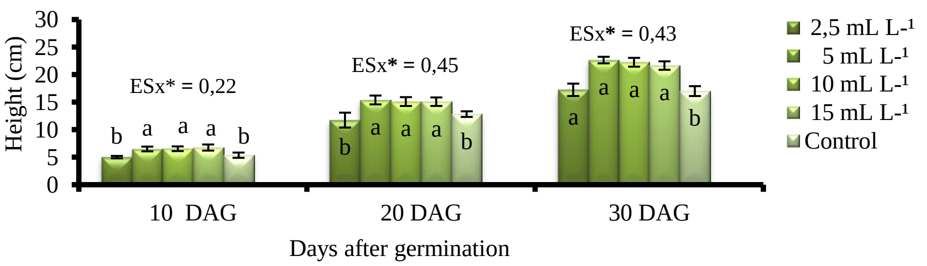
1. Imbibition 2.5 mL L^{-1}
2. Imbibition 5 mL L^{-1}
3. Imbibition 10 mL L^{-1}
4. Imbibition 15 mL L^{-1}
6. Control without bioproduct

The tomato seeds were soaked for 15 minutes. Each treatment occupied an experimental surface of 2 m^2. The sowing was done with a stream with a density of approximately 250 seeds per m^2, with a distance between rows of 10 cm. The data were processed through the Statgraphics Centurión program (version 15.1). The cultural attentions to the seedbed were carried out according to the norms established in the Technical Manual of the crop. Growth evaluations were carried out on 10 plants per treatment taken at random at 10, 20 and 30 days after germination (DAG), they are shown below:

- ✓ Height (cm): with a graduated ruler, it was measured from the root neck to the axilla of the youngest leaf.
- ✓ Root length (cm): with a graduated ruler, the main root of the crop was measured.
- ✓ Number of leaves: visual count
- ✓ Stem diameter (cm): with a Vernier caliper, it was measured from two centimeters from the root neck. (it was evaluated only at 20 and 30 DAG)
- ✓ Foliar and radical dry mass (g): by weighing in an analytical balance with an accuracy of ± 0.01 mg and drying in an oven at 70 °C until constant mass.

RESULTS AND DISCUSSION

In Figure 1, the height dynamics of the tomato plants are shown, at 10, 20 and 30 days after germination. At each evaluative moment it was found that, the treatments embedded in the highest dilutions (5, 10 and 15 mL L^{-1}) did not present significant differences between them. However, they statistically exceed the lowest dilution (2.5 mL L^{-1}) showing that it is necessary to evaluate a range of concentrations of the bioproduct from 5 to 15 ml L L^{-1} and determine the most appropriate ones, capable of stimulating the height of the plant.



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

Figure 1. Effect of the imbibition of tomato seeds in different dilutions of MI on the height of the plant at different moments of evaluation

The height of the plant reached with the highest concentrations studied (5, 10 and 15 mL L^{-1}) values of around 6.75 cm at 10 days after germination, which represents an increase with respect to the control in 25.9 %. In subsequent evaluations at 20 and 30 DAG, a similar behavior was maintained with values close to 15 and 20 cm, increasing with respect to the control by 20 and 33 % respectively. Among the growth and development variables of the tomato crop, it is considered that height is the one that most influences yield [13].

According to the technical instructions for the culture, the optimal length required for transplantation is 15 cm, which was reached in the aforementioned treatments at approximately 20 days, it did not occur in the same way with the control and the 2.5 mL L^{-1} dilution, which they achieved values within the quality parameters to be transplanted around 30 days. It constituted a 10-day decrease in the time of permanence of the seedlings. The imbibition of the seeds in different dilutions of the MI represents an alternative for the production of seedlings with the required height in less time.

The behavior presented by the root length is shown in (Figure 2). The analysis of the length of the root referred a similar behavior to the variable height of the plant where, with the exception of the dilution of 2.5 mL L^{-1}, the rest stimulates the root length without statistical differences between them.

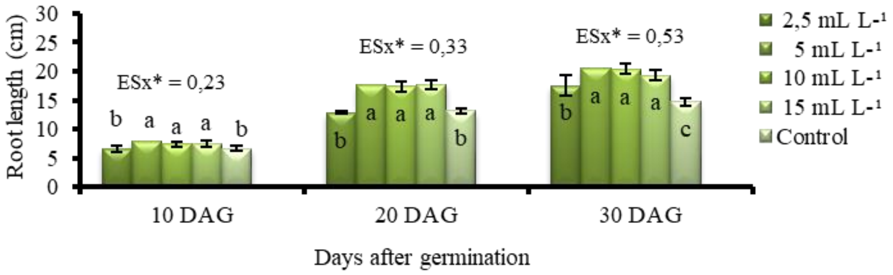


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Figure 2. Effect of the imbibition of tomato seeds in different dilutions of MI on the length of the root

The behavior presented by the root length could be associated with the physiological activity of the inoculated microorganisms. The well-developed root system can contribute to the absorption of greater amounts of water and nutrients. Several studies emphasize the role of *Bacillus* in the secretion of radical exudates that take place from the water absorption phase of the germination process until the end of its biological cycle. It causes temporary disturbances in the membrane and consequently a loss to the surrounding environment of solutes and different low molecular weight metabolites (sugars, organic acids, ions, amino acids, polypeptides among others). It can be used by the inoculated microorganisms and therefore an increase in the production of the phytohormone indoleacetic acid (IAA), a plant growth promoter compound, is obtained, which induces an increase in the number and length of root hairs [14,15].

Studies carried out by the ICIDCA report that this MI is capable of producing between 14 and 18 % of IAA, an aspect of vital importance for its application in agriculture as a biostimulant [7].

This could explain the appreciable effect of dilutions with respect to the control, statistically increasing the results of the variables studied.

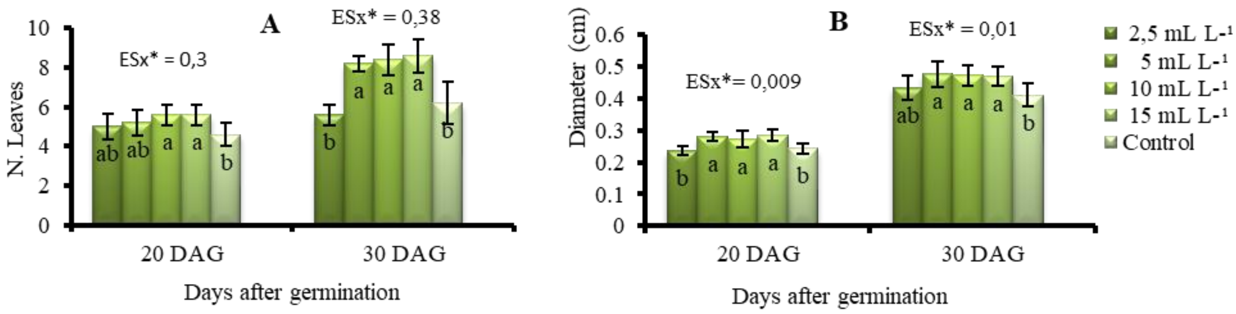
Tomato seeds, when hydrated under conditions of partial anoxia, present, like the generality of all crops, a triphasic pattern of water absorption, where phase I is characterized by rapid imbibition, due to purely physical factors, in particular the component matric of the water potential of the tissues. Phase II is generally a long period of water absorption and is mainly associated with metabolic events related to germination. The last stage (phase III) is associated with the emergence of the radicle, which constitutes the first visible sign of the germination process. According to these authors, root emission occurs at approximately 48 hours [(16)].

Therefore, according to what has been stated, it can be interpreted that the dilutions used could have decreased the frequency of these phases, causing an acceleration in root emergence and consequently an increase in radical growth with respect to the control. ICIDCA researchers presented similar results when evaluating different dilutions (5, 10, 15, 30, 75 and 125 mL L^{-1}) of IM in the germination of chard seeds (*Beta vulgaris* L.). All the dilutions evaluated stimulated the germination and growth indicators of the seedlings, decreasing their effect as the concentrations increase. It reaches a germination index (GI) for 125 mL L^{-1} (GI) $\leq$ 30 %, considered very low, which can be explained by the hormesis phenomenon, where the lower the dose, the greater the stimulation and the greater the inhibition, the higher the dose [(7)].

Root hairs, in addition to absorbing water, play a very important role in fixing the young primary roots as they penetrate the soil. The rhizosphere can indirectly influence the absorption of water and nutrients to maintain the turgor of the aerial part and enable biochemical activities [(17)].

The imbibition in the product benefited the increase in the size of the root, which allows us to infer that this in turn had a determining effect on the development of the aerial part of the plants, because of the efficiency of photosynthesis and nutrition. mineral. The imbibition of the seeds in different dilutions of the IM represents an alternative for the production of seedlings with the height and root length required in less time.

In Figure 3 A and B the effect of inoculation is observed both for the variables number of leaves and for the diameter of the stem. Both figures show the last two evaluative moments (20 and 30 DAG); the evaluation of the number of leaves, carried out at 10 DAG, it is not represented in the graph, since it did not show significant differences; what could be given by the beginning of the emission of its first leaves with photosynthetic capacity.



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

Figure 3. (A and B): Effect of the imbibition of tomato seeds in four dilutions of the MI on the Number of leaves (A) and the diameter of the stem (B) at different evaluation moments

When analyzing these variables together, it can be seen that in both evaluations there are significant differences between the treatments, with a tendency to increase with the application of the highest dilutions, (5, 10 and 15 mL L^{-1}) which do not differ significantly from each other. For both variables, the lowest values correspond to the control treatment. The results at 20 DAG show that the development of the postures was favored with the application of the product in 22 and 16 % for the leaves and the diameter respectively. This last variable managed to reach at this evaluative moment with the dilutions of 5, 10 and 15 mL L^{-1} values of diameter between 0.27 and 0.28 cm, close to 0.3 cm value established by the Technical Instruction of the culture as optimal for transplantation.

On the other hand, with 5 mL L^{-1} at 30 DAG, the stimulating effect of this product is also manifested both for the number of leaves and for the diameter of the stem; both were superior to the control in 32 and 16 % respectively. The increase in both variables is important since the seedlings will have a larger leaf surface to carry out photosynthesis, which gives them a greater ability to survive in the field.

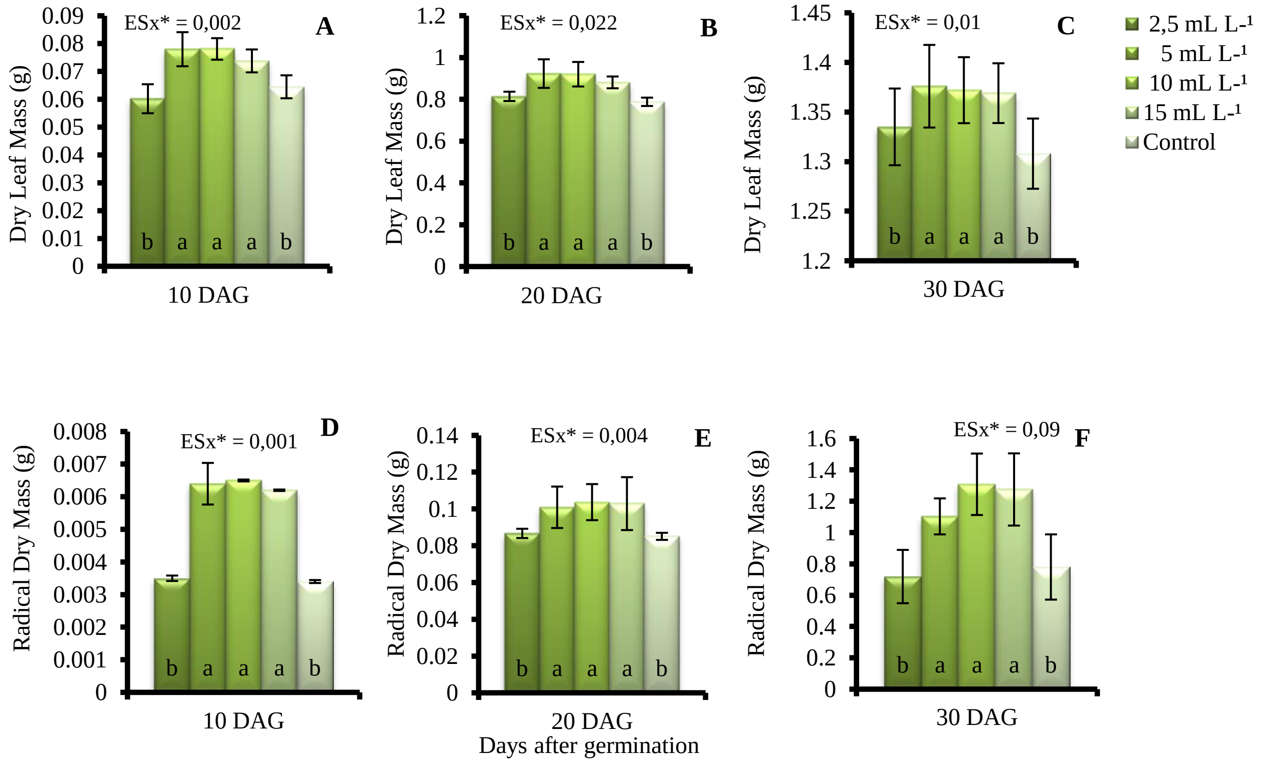
The growth of the stems requires the contribution of the photoassimilates obtained in the leaves that move through the phloem towards the consumption sites. The movement of these solutes occurs by mass flow from the source (leaves) to the sink (stems). For an efficient process of discharge of photoassimilates from the leaves to the stems, the continuous circulation of water between the xylem and the phloem is essential. Therefore, the increase in these indicators can influence the accumulation of foliar dry mass because of the balance between photosynthesis and respiration [(18)].

Research carried out shows that efficient microorganisms, when they are exposed to organic matter, secrete beneficial substances such as vitamins, organic acids, minerals, and antioxidants, change the micro and macro flora of the soil and improve the natural balance.

The antioxidant effects promote the breakdown of organic matter and increase the humus content, all of which promote plant growth. In relation to this approach, some authors suggest that EM degrade complex proteins and carbohydrates, in addition to producing bioactive substances (vitamins, hormones, enzymes) that can stimulate the growth and activity of other species of microorganisms. This could explain the increase in the number of leaves and stem diameter obtained in this experiment [(19)].

The interpretation of the results obtained in relation to the foliar and root dry mass of the plants, showed in both cases that the highest increases were obtained with the dilutions 5, 10 and 15 mL L^{-1}, treatments that do not differ significantly between them (Figure 4).

The opening or closing of the stomata is a determining aspect in the accumulation of carbon and biomass in plants, and it is closely related to the increase in leaf area. The flow of CO_2 to the photosynthetic sites occurs through the stomata. If the entry of CO_2 is limited because of the stomatal closure, the foliar area is reduced and therefore the dry mass production of the organs in the plants tends to decrease. Considering these criteria, it could be that the increase of these indicators in the plants of the aforementioned treatments has increased the flow of CO_2 and therefore greater accumulation of foliar and radical dry mass [(20)].



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

Figure 4. (A, B, C, D, E and F): Effect of the imbibition of tomato seeds in different dilutions of IM on the Foliar Dry Mass (A, B and C) and the Radical Dry Mass (D, E and F) at different evaluative moments

It should be noted that the imbibition could have led to an effective inoculation of the seeds, which clearly shows the differences with the control treatment, in this regard a similar behavior of the control was noted in all the variables studied and in each of the evaluative moments, always below the studied treatments. Similar results when evaluating the behavior of the simple and combined foliar spraying of 10 mL L^{-1} of the IM + AMF in the pepper crop (*Capsicum annuum* L.), where the height of the plants was also significantly higher, in the coinoculated treatment, even though it did not differ from IM treatment. They also reported significant results with the foliar spraying of 10 mL L^{-1} of the IM at 10 and 20 DAG in different horticultural crops (8).

Inoculation with efficient microorganisms (EM) to the ecosystem can improve the quality of the soils, as well as the growth, yield and properties of the crops. Studies carried out with the use of efficient microorganisms in tomato cultivation; manage to stimulate the growth and yield of the plants with significant statistical differences in relation to the control treatments. When evaluating the agro-productive behavior of *Zea mays* inoculated with Efficient Microorganisms, refers an increase with respect to the control, of the number of ears per plant, mass of the ears with straw and mass of the ear without straw of 15.8, 14.9 and 29.8 % respectively (21–24).

Published studies demonstrated the positive effect of 10 mL L^{-1} of the IM when evaluating some components of the yield in vegetables, finding that the mass and the equatorial diameter of *Brassica oleracea*, achieved a significant increase of 16 and 6% respectively with respect to the control. In addition, the cultivation of *Lactuca sativa* and *Beta vulgaris* increased their mass with respect to the control without bioproduct in 68 and 72 % (8).

IM contains the bacterium *Bacillus subtilis* (Plant Growth Promoting Rhizobacteria) which is capable of secreting plant growth regulating substances. The best known are phytohormones, substances with high biological activity that, in small concentrations, influence the metabolism of plants, causing variations in their growth and development (25).

The fact that the microorganisms in these treatments have survived and adapted shows that the environment of the rhizosphere where the research was carried out has favorably influenced them. As has been reiterated in previous sections and according to the criteria of other authors, due to soil conditions, microorganisms can dominate or coexist and take forms of compensation in the present microbial context (26).

On the other hand, of the four dilutions studied in the nursery, three of them (5, 10, 15 mL L^{-1}) were able to significantly stimulate the growth indicators of the plants with respect to the control at 10, 20 and 30 DAS, allowing produce plants in less time and with the necessary quality.

However, just soaking the seeds in 5 mL L^{-1} for 15 minutes produced a significant saving of the product an element that from a practical point of view influenced the selection of the dilutions used.

CONCLUSION

The microbial inoculum based on Efficient Microorganisms stimulates the growth and development of tomato plants in the seedling phase, the best combination resulting in soaking them for 15 minutes in the dilution of 5 mL L^{-1}.

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