IECAG 2021

MDPI

Proceedings

Effect of *Rhizobium* inoculation on tomato (*Solanum lycopersicum* L.) yield in protected crops.

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†Presented at the 1st International Electronic Conference on Agronomy, 3–17 May 2021; Available online: https://sciforum.net/conference/IECAG2021

Abstract: The insufficient availability of nutrients in the soil and the non-use of biofertilizers as a strategy in the tomato nutrition process are factors that limit the yield of this crop. The objective of this research was to evaluate the effect of different Rhizobium strains on the yield of the Aegean hybrid tomato variety. The inoculation of the microorganisms was carried out at the time of sowing and transplantation, in a proportion of 10% with respect to the volume of the root ball. The experimental design was in randomized blocks, with four treatments and each one with four replications, an uninoculated control and three levels of the inoculation factor with the strains of *Rhizobium*, *Rhizobium etli* CE-3, *Rhizobium leguminosarum* SCR; *Rhizobium leguminosarum* Semia-4088. The sampling was carried out in zig zag throughout the field and the following variables were evaluated: dry mass by plant organs, foliar NPK, growth indicators, productive indicators, crop yield and economic evaluation. The results achieved showed a positive effect on the indicators evaluated in the plants inoculated with the Rhizobium strains with respect to the control without inoculation. With the inoculation of the *Rhizobium etli* CE-3 strain, the best results were obtained in tomato yield.

Keywords: tomato; inoculation; rhizobium; symbiosis; yield.

Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *Proceedings* **2021**, *68*, x. https://doi.org/10.3390/xxxxx

Published: date

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important and in-demand horticultural crops in Cuba, this is due to its excellent nutritional properties and its role in the eating habits of a large part of the population, both for fresh consumption and However, its performance is limited by different factors, the highest incidence being: the inappropriate use of chemical fertilizers and the insufficient availability of nutrients in the soil, mainly nitrogen, affecting the growth and production of this crop.

In Cuba, climatic changes hinder the productivity of this vegetable during much of the year. In this sense, greenhouse tomato cultivation occupies the largest cultivated area, which allows protecting the crop from adverse conditions such as high temperatures, light intensity, incidence of rain, wind and insect attack, with the aim of obtaining crops in non-optimal periods for this vegetable [1]. In 2018, the tomato production in Cuba was 43,405 t, in the Santiago de Cuba province a production of 365.3t is reported [2,3]. In the protected cultivation unit "Campo Antena" in the same year the average yield of the Aegean hybrid variety was 60t / ha-1.

Among the factors that influence the decrease in the production of the tomato crop, the inappropriate use of chemical fertilizers is the one that most influences the growing deterioration of biodiversity and soil balance, whose damages are sometimes observed in the long term [4]. For this reason, it is currently necessary as one of the most valuable elements to consider, to promote sustainable agriculture from the use of biofertilizers, which allows reducing the use of chemical fertilizers, improving the absorption and availability of nutrients in the soil and with this, promote new production systems that increase yields and generate excellent quality products that guarantee agricultural development without contaminating the ecosystem, preserving soil fertility and biodiversity.

Associated with the rhizosphere, various microorganisms reside, whose ability to promote the growth of crops of interest, favor the supply of nutrients to the soil or plants, can be exploited as a sustainable strategy to increase productivity. Within these microbial groups, the plant growth promoting bacteria (PGPR) stand out as essential elements, which act in a coordinated way at the soil-root interface, this group of bacteria includes the genus *Rhizobium* which has been widely studied in recent years in order to check whether nitrogen fixation is feasible in non-legume plants [5]. Among the described biochemical mechanisms exerted by (PGPR) and that have beneficial effects on plants is the biological fixation of atmospheric nitrogen (BNF), carried out by symbiotic rhizobacteria such as *Rhizobium* sp. or others of free life like *Azotobacter* sp. and *Azospirillum* sp. that have been used extensively as biofertilizers to improve nitrogen availability in vegetables such as tomato (*Solanum lycopersicum* L), onion (*Allium cepa* L.) and lettuce (*Lactuca sativa* L.) [6-8], other mechanisms is the solubilization of phosphorus (P), the synthesis of phytohormones, vitamins and enzymes, which allows reducing the incidence of diseases and pathogens as well as greater tolerance to abiotic stress, increased absorption of water and nutrients [5,9].

In the protected crops unit "Campo Antena" belonging to the Empresa Integral Agropecuaria Santiago de Cuba, prior to the investigation, evaluations of chemical analysis were carried out on the soil, the insufficient availability of nutrients was determined, causing a decrease in the productivity of the cultivation of tomatoes. Due to the above, the objective of this research is to evaluate the effect of different strains of Rhizobium on tomato yield (*Solanum lycopersicum* L.).

2. Materials and methods

2.1. Location and conditions of the experiment.

The research was development in the Protected Crop Unit "Campo Antena", coordinates X: 607547.321; Y: 156420.837, belonging to the Empresa Integral Agropecuaria Santiago de Cuba, from November 2018 to April 2019 on a brown soil without carbonates [10]. The chemical and microbiological analyzes shown in table 1 were performed in the Laboratory of Soils, Plants and Waters, of the Department of Biofertilizers and Plant Nutrition, of the National Institute of Agricultural Sciences (INCA).

Table 1. Chemical and microbiological characteristics of the arable soil layer (0-20cm deep).

pH en (H_2O)	**MO (%)**	**P (mg Kg^{-1})**	**Na^+**	**K^+**	**Ca^{2+}**	**Mg^{2+}**
			($cmol_c\ Kg^{-1}$)			
7.25	2.69	182.7	1.03	2.09	26.5	13.2
Microbiological analysis of the soil.						
No. native rhizobia: $1{,}8x10^5$UFC g^{-1}						

Chemical determinations: pH in H2O by potentiometer method: soil / solution ratio of 1: 2.5; MO (organic matter) Walkley and Black P: 0.1 N H2SO4 solution with soil-solution ratio 1: 2.5, NH4Ac cations at pH 7 [11].

2.2. Description of the experimental design, experimental area and applied treatments.

During the investigation, a completely randomized experimental design was used, with an experiment with four treatments and each treatment with four replications. Four houses of protected cultivation of 0.08 ha^{-1} were used for a total experimental area of 0.32 ha^{-1}, these houses had analogous conditions for the experiment. The number of tomatoes beds per house is 10 and the number of rows per tomatoe beds is one, the planting frame 1.04 m x 0.40 m and the number of plants per house is 1923. The crop under study was tomato Hybrid Aegean variety. The stage to be evaluated was from transplantation to final production. The treatments applied in the protected cultivation houses were: (T1) control without inoculation, another three inoculation factors with the strains (T2) *Rhizobium etli* CE-3, (T3) *Rhizobium leguminosarum* SCR, and (T4) *Rhizobium leguminosarum* Semia- 4088.

2.3. Selection of Rhizobium strains and method of inoculation at the time of transplantation.

Before selecting the strains, the native rhizobia colony forming units (CFU mL^{-1}) were counted. This sampling was carried out in a zig zag manner in the four cultivation houses where the experiment was developed To determine the number (CFU) of rhizobia, it was performed by serial dilutions of 1g of soil in 9 mL of sterile distilled water, which were seeded on Petri dishes with Mannitol Yeast Agar medium, and incubated at 30 ° C for 7 days. [12]. The strains used are from the stock of the Microbiology laboratory, Department of Plant Physiology and Biochemistry of INCA, from which certified inoculated were obtained in medium, with a concentration of 10^8 CFU mL^{-1}. At the time of sowing, a 200 mL dosage of each *Rhizobium* strain was applied for every 50 kg of seeds as recommended by INCA, adapting the dose to the crop, at the time of transplantation, 30-day sowing positions were used with a mean height of 12 cm, 3 pairs of true leaves and a thickness of the stem of 4.2 mm, the strains were used in relation to the volume of the root ball, applying in each treatment a proportion of 10% of the covering of the root balls. The next day after transplanting, a light irrigation of 0.5 liters per plant was applied with acidified water at a dosage of 136 mL of H_3PO_4 at 85%, and 40 g of Premium Chelate per m^3 of irrigation water. The management of the plantation was carried out taking into account the technology of the crop and the biotic and abiotic conditions in which they were developed.

2.4. Variables evaluated

2.4.1. Variables of the growth and development of the plant.

These evaluations were made in 10 plants per replicate for a total of 40 per treatment, the evaluations were made 25, 50 and 75 days after the definitive transplant (d.a.t.). The height measurements were made from the base to the last leaf sprout at the apex of the main stem, with the help of a tape measure (Hunter brand 3m 10ft x 16mm) and for the diameter of the stem they were made at its base, with the help of the Caliper, (Mitutoyo brand 530 - 114 - 200 mm).

2.4.2. Variables dry mass by plant organs (g plant-1) and foliar NPK.

They were carried out in the harvest phase of the third cluster to harvest of the third to last cluster, 5 plants were taken for each treatment, for this each organ was weighed separately on a Sartorius digital balance BSA 124S Max 120g. They were dried in a Boxun BGZ oven at 70 ºC, for 48 hours and each sample was weighed with an interval of 2 hours until reaching a constant mass, determining the dry mass of each one by difference. The foliar NPK analysis was carried out in the Soil, Plant and Water Laboratory, of the Department of Biofertilizers and Plant Nutrition, of the National Institute of Agricultural Sciences (INCA) [11].

2.4.3. Productive and yield variables.

Regarding the productive and yield variables, 40 fruits were chosen at random for each treatment throughout the productive cycle. The average equatorial diameter (cm) and fresh mass (g fruit-1) of the fruits were made with the help of the Caliper and a Sartorius digital BSA 124S Max 320g scale respectively. To find the yield of each treatment, the total production of each experimental plot was divided by the total area.

2.4.4. Economic evaluation

It was analyzed taking into account the production cost (CP) in $/ha (direct and indirect), production value PV ($/ha) calculating the yield for the sale price according to the national price list of protected crops for facilities state, Profit (P) in $/ha was determined value of production less cost of production and profitability (P) by means of the profit between production cost. [13].

2.5. Statistic analysis.

The experimental data for each variable studied were subjected to a simple classification analysis of variance (ANOVA), when there were significant differences. Comparisons of means were made according to Duncan's multiple range test for $p \leq 0.05$. The results were evaluated using the statistical package Stagraphics Centurion. XV.v15.2.14 and were graphed with the Microsoft Excel 2010 program.

3. Results

3.2. Variables of the growth and development of the plant.

Table 2 shows the variables of average height and thickness of the tomato plants evaluated at 25, 50 and 75 days after transplantation pre-inoculated with the *Rhizobium* strains under protected conditions, the evaluated variables showed a greater increase in the average height and thickness of the plants inoculated with *R. etli* CE-3 and *R.l*-SCR in comparison with the other strain and the control treatment, which at the moments evaluated at 25 and 75 (d.a.t.) did not show significant differences between them as well as in the thickness of the stem in the first evaluation.

Table 2. Height (m) and Thickness (mm) of the plant inoculated with *Rhizobium*.

Treatments	**First measurement (25 d.a.t.)**		**Second measurement (50 d.a.t.)**		**Third measurement (75 d.a.t.)**	
	Height (m)	Thickness (mm)	Height (m)	Thickness (mm)	Height (m)	Thickness (mm)
(Control) not inoculated	0.27c	10.8c	0.69d	14.3d	1.02c	18.9d
R. etli CE-3	0.43a	12.1a	0.84a	16.5a	1.21a	21.3a
R.l-SCR	0.36b	11.7b	0.77b	15.8b	1.13b	19.7b
R.l Semia-4048	0,31bc	11.2bc	0.73c	15.1c	1.07c	19.1c
ESM	0.0105	0.0807	0.0451	0.116	0.033	0.126

T1 (Control) not inoculated; T2 (*R.etli CE–3),* T3 (*R.l SCR), T4 (R.l Semia-4048).* Means with different letters have significant differences ($p \leq 0.05$).

3.3. Variable Dry mass by plant organs and Foliar NPK content.

Table 3. Dry mass (g plant^{-1}) and NPK content (g kg^{-1}) foliar inoculated with *Rhizobium*.

Treatments	**Dry mass (g plant^{-1})**			**NPK(g kg^{-1}) foliar**		
	Leaf	Stem	Root	N	P (P_2O_5)	K (K_2O)

(Control) not inoculated	10.35d	3.84d	1.62d	2.585d	0.105c	0.595c
R.e CE-3	18.69a	6.65a	3.41a	3.597a	0.187a	0.823a
R.l SCR	16.08b	5.02b	2.18b	3.285b	0.125b	0.685b
R.l Semia-4048	11.00c	4.40c	2.05c	3.012c	0.108b	0.678b
ESM	0.1067	0.3431	0.1167	0.1124	0.0436	0.0253

T1 (Control) not inoculated; T2 (*R. etli CE–3),* T3 (*R.l SCR), T4 (R.l Semia-4048).* Means with different letters have significant differences (p≤ 0.05).

The results of the dry mass per organs of the tomato plants inoculated with *Rhizobium* at 80 days after transplantation show significant differences between the treatments and the organs of the evaluated plants. The highest dry mass values were evidenced in the leaves with the treatment inoculated with the *R. etli* CE-3 strain showing the best results. For the foliar contents of NPK present in the plants inoculated with *Rhizobium*, the T2 (*R. etli* CE – 3) obtained the best results, the statistical analysis showed significant differences between the treatments for N; the P and K values did not show significant differences for the treatments T3 (*R.l* SCR), T4 (*R.l* Semia-4048) but the results were superior with respect to the production control.

3.4. Crop yield variable.

Table 4. Equatorial diameter (cm), weight (g) of the fruits and Yield in (t / ha^{-1}) inoculated with *Rhizobium*.

Treatments	Equatorial diameter (cm)	Fruit weight (g)	Yield (t/ha^{-1})
(Control) not inoculated	5.1d	141.33d	70.20d
R. etli CE-3	7.9a	235.25a	81.16a
R.l SCR	6.5b	155.43b	77.55b
R.l Semia-4048	5.8c	150.36c	72.25c
ESM	0.1426	0.3261	0.1943

T1 (Control) not inoculated; T2 (*R.etli CE–3),* T3 (*R.l SCR), T4 (R.l Semia-4048).* Means with different letters have significant differences (p≤ 0.05).

When analyzing the variables of the crop yield: equatorial diameter and weight of the fruits, it was observed that the highest values were registered in treatments 2 and 3 inoculated with *R. etli* CE-3 and *R.l* SCR respectively, in the same way occurred in the yield reaching values 81.16 and 77.25 t / ha-^{1} in the same order.

3.5. Economic evaluation.

Table 5. Economic evaluation Thousands of pesos Cuban currency thousands.

Treatments	Economic indicators in Cuban currency thousands.			
	C.P	VP	P	P
(Control) not inoculated	10.33	16.8	6.47	0.62
R.etli CE-3	10.41	25.92	15.51	1.48
R.l SCR	10.39	24.88	14.49	1.39
R.l Semia-4048	10.39	23.12	12.73	1.22

T1 (Control) not inoculated; T2 (*R.etli CE–3),* T3 (*R.l SCR), T4 (R.l Semia-4048).* Means with different letters have significant differences (p≤ 0.05). C.P (Cost of production), VP (Value of production), P (Profit), P (Profitability).

Table 5 shows the economic results of the evaluation of Rhizobium in tomato cultivation. The evaluated treatments showed significant differences, but treatment two in-

oculated with R. etli CE-3 was the one that performed the best with respect to the other two strains evaluated and the control without inoculation with a profitability of 1.48. In this experiment, no monetary losses were quantified despite the fact that the evaluated treatments did not behave in the same way.

4. Discussion

In the research, the results obtained from the variables evaluated show the efficiency of *Rhizobium* and the strains used. The treatments inoculated with the *R. etli* CE-3 and *R.l* SCR strains were the ones that achieved the best results with respect to the evaluated *R.l* Semia-4048 strain and the uninoculated control. The results obtained show the positive effect of applying Rhizobium strains to tomato plants.

In previous research (regardless of the methodology) they have described that the inoculation of Rhizobium has managed to improve the growth and development of tomato (*Solanum lycopersicum* L) seedlings and refer to the fact that *Rhizobium* is a microorganism capable of fixing nitrogen asimbiotically and dissolve phosphates favoring the nutrition of the tomato seedling, a quality that makes it a microorganism with PGPR capacity. [6], in the cultivation of lettuce (*Lactuca sativa* L.) positive results were obtained in the variables: dry weight of leaves, stems and shoots as well as in the length of the root and height of the plant with the application of the strain *R. etli* [8].

The results obtained in this investigation for each evaluated variable could be given by the capacity of these Rhizobacteria, which when interacting with the roots of non-legume seedlings are attracted by substances emitted by the root, allowing the movement of the bacteria towards the root of the plant seedling initiating a beneficial symbiosis, a process that occurs through chemotaxis mechanisms related to the presence of flagella, chemoreceptors and genetically encoded regulatory systems [14]. Other benefits that are conferred to Rhizobium is the direct action it exerts in the production of phytohormones, a process that occurs naturally. These phytohormones include five known groups of compounds: auxins, ethylene, gibberellins, cytokines, and abscisic acid, each of which has a direct action on plant growth and development [9].

When analyzing the data of the evaluated variables, it is possible that this rhizobacterium has produced giberillin capable of increasing plant growth and auxin, which is indo acetic acid (IAA), a phytohormone that its division at the cellular level facilitates an increase in size. of the fruits and the number of leaves. [14]. These growth regulating substances stimulate the density and length of the root hairs and lateral roots, thus developing water and nutrient absorption capacity that is evidenced in the results of the evaluated variables, achieving a beneficial effect on the dry weight of the aerial part crop yield [15], these results were corroborated by statistical analysis that yielded a significant difference ($p < 0.05$), so it could be stated that the *R.etli* CE-03 strain produced these phytohormones. The use of biofertilizers allows working on a sustainable agriculture approach, based on the use of beneficial microorganisms, which can guarantee high yields of agricultural crops with lower costs, a higher biological quality of crops, increased biological activity of the soil, depending on the care of the environment.

5. Conclusions

The results obtained showed a positive effect on the indicators evaluated in the plants inoculated with the Rhizobium strains with respect to the control without inoculation. With the inoculation of the *R.etli* CE 3 strain, the best results were obtained in tomato yield (*Solanum lycopersicum* L.).

Funding: This research did not receive external funding.

Acknowledgments: Special thanks to the management of the Empresa de Proyectos e Ingeniería (Project an Engineering Company) (ENPA) and the University de Oriente for

their wonderful support and contribution in carrying out this project. To the colleagues of the protected cultivation unit Campo Antena, for all their support in carrying out this research. To the colleagues of INCA for their support in the analysis of the samples taken in the field, contribution of equipment, trained personnel and excellent technical assistance.

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