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Effect of a Marine Bacterium and Vermicompost on Antioxidant Properties and Fruit Quality of *Solanum lycopersicum* L.
Efecto de una Bacteria Marina y Vermicomposta sobre las Propiedades Antioxidantes y la Calidad del Fruto de *Solanum lycopersicum* L.

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SUMMARY

The present study aimed to evaluate caporal hybrid tomatoes' antioxidant capacity and fruit quality under greenhouse conditions when applying a combination of a marine bacteria (*Stenotrophomonas rhizophila)* and different doses of vermicompost. A random block design was used, with 12 treatments: Inorganic fertilized (IF, control treatment), *S. rhizophila* (Sr), 60, 150, and 300 g of vermicompost (V), and the combination of Sr+V (Sr+V60, Sr+V150, and Sr+V300), with 20 repetitions each. Plant height, stem diameter, number of flowers, number of fruits, colony-forming units (CFU), polar and equatorial diameter of the fruit, fruit firmness, titratable acidity, total soluble solids (TSS), lycopene, vitamin C, fruit weight and yield per plant were evaluated. These were analysed with the Statistical Package and the Tukey mean test with a significance level of 0.05%. The analysis showed significant differences for all the variables evaluated with Sr+V300 as the best treatment. It is concluded that the use of bacterium isolated from marine environments in combination with vermicompost favors the antioxidant contents and fruit yield being an alternative use in this vegetable.

Index words: *lycopene, Stenotrophomonas rhizophila, vitamin C, yield per plant.*

RESUMEN

El presente estudio tuvo como objetivo evaluar la capacidad antioxidante y la calidad del fruto de tomate en condiciones de invernadero al aplicar la combinación de una bacteria marina (*Stenotrophomonas rhizophila*) y diferentes dosis de vermicomposta. Se utilizó un diseño de bloques al azar con 12 tratamientos: fertilizado inorgánico (IF, tratamiento control), *S. rhizophila* (Sr), 60, 150 y 300 g de vermicompost (V) y la combinación de Sr+V (Sr+V60, Sr+V150 y Sr+V300) con 20 repeticiones por tratamiento. Se cuantifico: altura de la planta, diámetro del tallo, número de flores, número de frutos, unidades formadoras de colonias (UFC), diámetro polar y ecuatorial del fruto, firmeza del fruto, acidez titulable, sólidos solubles totales (SST), licopeno, vitamina C, peso del fruto y rendimiento por planta. Se utilizó un paquete estadístico y la prueba de medias de Tukey con un nivel de significancia del 0.05%. El análisis mostró diferencias significativas para todas las variables evaluadas con Sr+V300 como el mejor tratamiento. Se concluye que el uso de la bacteria aislada del ambiente marino en combinación con vermicomposta favorece el contenido de antioxidantes y el rendimiento de fruto en esta hortaliza.

Palabras clave: *licopeno, Stenotrophomonas rhizophila, vitamina C, rendimiento por planta.*

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop in the world after the potato (*Solanum tuberosum* L.) and has registered an annual global yield of approximately 170.75 million tons produced in 5.02 million hectares planted in more than 150 countries (Zamora-Oduardo *et al*., 2020; FIRA, 2019). In Mexico, the production of this nightshade in that year was 546 322 tons, with a planting area of 23 960 hectares and a production of one million 154 thousand 562 tons (SIAP, 2021).

However, to maintain desired productivity levels, the conventional and excessive application of chemical inputs usually resorts, which increases production costs and gives rise to a series of environmental and human health problems (Yahaya, Mahmud, Abdullahi, and Haruna, 2023; Liu, Zheng, Wei, Kai, and Xu, 2021).

This background has motivated the search for alternatives that ensure the product's safety without diminishing the harvested fruits' yield and quality. The use of organic fertilizers, such as vermicompost, becomes an eligible alternative to the nutraceutical properties of the fruits (Andreu-Coll *et al*., 2023; Espinosa-Palomeque *et al*., 2020), as has been reported in melon (*Cucumis melo*) cv. 'Crusier' (Sánchez-Hernández *et al*., 2016), tomato (*S. lycopersicum*) (Loganathan, Garg, Venkataravanappa, Saha, and Rai, 2014; López-Martínez *et al*., 2016; Salas-Pérez *et al*., 2016, 2017; Fortis-Hernández *et al*., 2021), among others.

Moreover, microorganisms isolated from marine environments with characteristics that promote plant growth become one more option of eligibility not only to biostimulate and modulate the levels of endogenous phytohormones and influence their phenological behavior and development (Lara-Capistrán *et al*., 2020a) but also as a source of bioactive substances and various nutraceuticals that have numerous biological activities for humans and animals (Ande, Syamala, Srinivasa, Murali, and Lingam, 2017), which can be used as a functional food supplement (Gupta and Prakash, 2019).

Among these microorganisms, the marine bacteria produce versatile secondary metabolites, including lipopeptides, polypeptides, macrolactones, fatty acids, polyketides, and isocoumarins capable of acting on a wide range of antimicrobial, anticancer, and antialgal activities or as a pesticide and tool for bioremediation (Nguyen, Han, Kim, and Choi, 2022; Wang *et al*., 2022; Srinivasan, Kannappan, Shi, and Lin, 2021). In addition, marine strains are metabolically different from terrestrial species and, therefore, differ in their environment and the type of distinctive bioactive compounds they synthesize (Masís-Ramos, Meléndez, and Méndez, 2021; Eriksen *et al*., 2020).

Stenotrophomonas rhizophila is a marine bacterium which has demonstrated inhibition capacity towards various phytopathogens (Reyes-Peréz *et al*., 2019; Rivas-Garcia *et al*., 2019) and it has been characterized as a producer of phytohormones, siderophores, antibiotics, among others (Elhosieny, Zayed, Selim, Yassen, and Abdel, 2023; Raio *et al*., 2023; Hernandez-Montiel *et al*., 2017). However, little is known about the interaction that can exist when combined with vermicompost on fruit quality and antioxidant properties. Therefore, the present study evaluated the effect of a strain of *S. rhizophila* isolated from marine environments and doses of vermicompost on the growth, production, and antioxidant status of the fruit of *Solanum lycopersicum* L. in greenhouse conditions.

MATERIALS AND METHODS

Study Location

The work was carried out in a 250 m^2 zenith greenhouse with 6000 caliber drip irrigation belts connected to a 1/2 horsepower pump in Xalapa, Veracruz, Mexico, with the geographical coordinates19° 33″ 05.37′ N., 96° 56″ 40.64′ O, and 1428 meters of altitud.

Experimental Design

A random block design was used, with 12 treatments: Inorganic fertilized (If), *S. rhizophila* (Sr), 60 g vermicompost (V60), 150 g vermicompost (V150), 300 g vermicompost (V300), *S. rhizophila* + 60 g of vermicompost (Sr+V60), *S. rhizophila* + 150 g vermicompost (Sr+V150), and *S. rhizophila* + 300 g of vermicompost (Sr+V300), with 20 repetitions each. The inorganic fertilized treatment (If) was at 100% and the other treatments were fertilized at 50% of the used doses. Inorganic fertilization is carried out every 8 days after transplanting.

Substrate Disinfection and Bag Filling

A mixture of 50% soil, 25% sand, and 25% tepezil was used. It was disinfected with metam sodium in doses of 973.52 mL diluted in 200 L of water until it was completely soaked by the soil covered with plastic for 72 hours. After resting, it was uncovered and vacuumed until the soil stopped presenting the product's smell and was completely dry. Subsequently, a toxicity test was conducted, germinating lettuce var. Parris Island seeds in a petri dish. The result was 80% germination (Sobrero and Ronco 2008), indicating that the substrate was ready to fill 5-kilogram plastic bags.

Seedling Transplantation and Inoculation with *S. rhizophila*

Seedlings of the Caporal hybrid of the company Vilmorin of determined growth were used, which were transplanted 30 days after their emergence at approximately 15 cm in height. The bacterium *S. rhizophila* from marine environments was provided by the Biotecnología Microbiana Laboratory from Centro de Investigaciones Biológicas del Noroeste. The increase of the bacterium was made through a liquid medium TSB in an Erlenmeyer flask with constant agitation for 72 h at room temperature and cell suspension of *S. rhizophila* was adjusted at 1×10^8 cells ml^{-1} using a UV/Vis spectrophotometer at 660 nm and absorbance of 1 (Reyes-Peréz *et al*., 2019). At transplant, each plant was inoculated at the root periphery with 3 mL of the marine bacterium.

Plant Management in the Greenhouse

The activities carried out inside the greenhouse for cultivating tomatoes began with placing chromotropic traps at strategic points. It was carried out with the insecticide potassium soap NEEM extract and petroleum jelly at a rate of 1 mL L^{-1}, followed by placing rings to tutor plants 20 days after transplantation (DDT) and adding the axillary bud cut. Formation pruning was performed, and the plants were managed to a single stem.

Vermicompost Characteristics

The coffee pulp vermicompost from Terranova lombricultores contained: organic matter 84.5%, pH 7.4, organic phosphorus 0.1%, total phosphorus 0.25%, total nitrogen 3.9%, total potassium 2.1%, total calcium 1.7%, total magnesium 0.8%, C/N ratio 12.2 and fulvic acid contents 10.5% and humic acids 15.1% (Terra Nova, 2022).

Fertilization

The fertilization was done by applying the inorganic nutrient solution (Steiner, 1984). Which was prepared using highly soluble commercial fertilizers (potassium nitrate, calcium nitrate, manganese sulfate, ferrous sulfate, magnesium sulfate, copper and zinc sulfate, ammonium phosphate, and boric acid) with a pH of 6.5 and the electrical conductivity (EC) was 2.0 dS m^{-1} with water (Oliva-Llaven *et al*., 2010). This fertilization was complemented by the nutritional package of the company AgroScience (2018) for the cultivation of tomatoes.

Variables Evaluated

The variables evaluated were plant height (cm), stem diameter (mm), number of flowers at 60 days after transplantation (DDT), number of fruits 90 DDT, visual counts, colony-forming units (CFUs), using the method proposed by Glick, Patten, Holguin, and Penrose (1999). Polar diameter of the fruit (cm), equatorial diameter of the fruit (cm) with a vernier graduated in mm, firmness of the fruit, with a Wagner penetrometer (force dial FDK 30 lb 14 kg^{-1}) with the appropriate piston (2-8 mm in diameter), ensuring that the pressure indicator needle was marked 0. Then, pressure is exerted until the plunger is completely introduced into the pulp of each fruit, and the first value obtained is recorded, expressed in newton (N), for acidity and vitamin C samples of 10 mL of juice were taken in 10 fruits of the fourth bunch, harvested in full ripeness. Each sample was diluted in 100 mL of distilled water and titrated with NaOH 0.1 N up to a pH of 8.2, expressing the result as citric acid (%), total soluble solids expressed in Brix degrees, cut into segments, and macerated in a mortar with the help of a pistil. Then, a sample of the extract obtained with a pipette was taken to deposit it, drop by drop, in the prism of the Reichert Refractometer Model 137530LO and through the eyepiece measure and adjust the shadow at the midpoint of the cross to read the refractive index on the upper numerical scale. Of this, the observed value is noted in °Brix.

For lycopene, this was done at 120 DDT. 100 g of pulp of 10 tomato fruits of each treatment, harvested in full ripening, was used, and the sample of the fourth bunch was within 24 hours of having been harvested. Next, 5 mL of the acetone-n-hexane mixture was added in a 4:6 ratio. It was then centrifuged at 5000 rpm for 5 min and 4 °C; the supernatant was extracted and read in a visible light spectrophotometer at wavelengths of 453 nm, 505 nm, 645 nm, and 663 nm, using the acetone-n-hexane mixture as a target according to the methodology of Rosales (2008[1]). Lycopene concentrations were quantified using the following equations proposed by Nagata and Yamashita (1992) for antioxidants in tomato fruits.

$$\text{Lycopene } (\mu g\ ml^{-1}) = 0.0458\ A663 + 0.204\ A645 + 0.372\ A505 - 0.0806\ A453 \quad (1)$$

For vitamin C, it was titrated with iodine solution 0.1N until observing a change in coloration, expressing the result in milligrams 100 g^{-1} of fresh weight (mg 100 g^{-1}) (IPGRI, 1996), the weight of the fruit (g), with a balance in grams and yield per plant, was made by adding 5 cuts, this was done with balance in kilograms.

Statistical Analysis

Analysis of variance (ANOVA) was developed using Statistics v 12.0 software (IBM SPSS Statistics, 2004). First, the assumption of normal distribution and homogeneity of variance of the data was verified using the Kolmogorov-Smirnov and Bartlet tests, subsequently, Tukey's comparison of means at 0.05 percentage.

RESULTS AND DISCUSSION

Morphological Variables

Vermicompost or *S. rhizophila* significantly affected plant growth parameters. The application of 300 g of vermicompost (V300) and *S. rhizophila* (Sr) + V300 (treatment Sr+V300) increased plant height. In the stem diameter, the plants with V300 obtained the highest values. Finally, treatment Sr+V300 increased; the number of flowers and fruits, and colony-forming units (CFU) ($P \leq 0.001$) (Table 1).

Moreover, the ANOVA in variables that are related to the quality of the fruit was obtained for the polar and equatorial diameters (Figure 1), with a greater significance ($P \leq 0.001$) when the plants were inoculated with *S. rhizophila* and with 300 g of vermicompost (Sr+V300) concerning the use of inorganic fertilization (If) and with *S. rhizophila* (Sr) only. The treatment *S. rhizophila* (Sr) + V300 (Sr+V300) significantly increased the polar and equatorial diameter of fruits ($P \leq 0.001$) (Figure 1).

Table 1. Effect of treatments on plant morpho/physiology of S. lycopersicum type Saladette, hybrid Caporal

Treatment	Plant height stem	Diameter	Number of flowers	Number of fruits	CFU
	cm	mm	- - - - - - - - - - - - - - - - - U	- - - - - - - - - - - - - - - - -	
If	57.80±2.70 c	5.66±0.29 c	22.70±0.95 d	21.10±0.74 d	0±0e
Sr	52.55±5.11 d	4.76±0.47 d	25.40±0.84 c	22.70±0.82 c	12.60±1.26 c
V60	66.70±2.55 b	6.39±2.31 b	18.80±0.42 e	14.90±0.32 e	6.50±0.53 d
V150	60.70±0.92 b	6.13±0.94 b	13.60±1.90 f	10.20±1.03 g	11.60±0.52 c
V300	77.10±3.54 a	6.92±0.28 a	18.40±0.70 e	14.10±1.79 e	16.40±1.07 b
Sr+V60	55.80±3.27 d	6.32±0.59 b	30.30±2.36 b	25.90±0.99 b	15.60±2.27 b
Sr+V150	58.60±0.32 c	5.98±0.67 bc	12.90±1.73 f	12.30±0.42 f	11.80±0.92 c
Sr+V300	73.65±0.53 a	6.33±0.39 b	46.70±2.75 a	38.50±0.53 a	19.80±1.55 a
±EE	0.20	0.20	0.30	0.27	0.20
F	21.9	8.9	30.9	50.8	13.79

Different superscripts in the same column suggest significant differences according to Tukey for $P \leq 0.05$.

[1] Rosales, M. (2008). *Producción y calidad nutricional en frutos de tomate cereza cultivados en dos invernaderos mediterráneos experimentales: Respuestas metabólicas y fisiológicas*. Tesis para obtener el grado de Doctor en Ciencias. Facultad de Ciencias. Universidad de Granada. Granada, España.

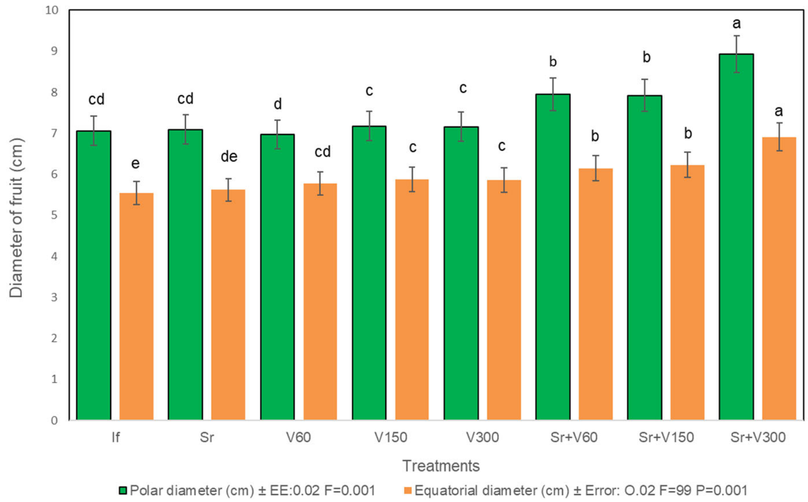

Figure 1. Effect of treatments on fruit morphology. Different superscripts represent significant differences according to Tukey, P ≤ 0.05. The vertical lines in the bars are the standard error (±).

In the increase in fruit quality (Table 2); firmness, total soluble solids (°Brix), and weight of the fruit (g) were favored with the application of 300 g of vermicompost and *S. rhizophila* (Sr+V300). The fruits of the fertilized plants presented the highest ($P \leq 0.001$) acidity percentages. In the lycopene, vitamin C, and yield, it was higher when the dose of 300 g of vermicompost waxs applied with *S. rhizophila* (Sr+V300) (Figure 2, 3, and 4, respectively).

The combination of *Stenotrophomonas rhizophila* isolated from the marine environment and high doses of vermicompost showed abilities to promote growth in tomato plants and improve fruit quality and yield parameters. As for the morphological parameters of the plants, *S. rhizophila* + 300 g of vermicompost (Sr+V300) turned out to be the best. This fact could be because one of the characteristics of incorporating vermicompost is to stimulate the populations of bacterial (Chakraborty, Saha, Dutta, and Sahu, 2023; Rehman, De Castro, Aprile, Benedetti, and Fanizzi, 2023) by increasing microbial activity (Enebe and Erasmus, 2023; Gupta and Prakash, 2019), a factor that influenced the effect of vermicompost and *S. rhizophila*, it may also be due to the content of vermicompost in macro and micronutrients (Maji, Misra, Singh, and Kalra, 2017) in addition to growth regulators such as auxins, gibberellins, cytokinins, fulvic acid and humic acid (Sengupta, Bhattacharyya, Mandal, and Chattopadhyay, 2022; Ravindran *et al*., 2019) that are used by plants as an energy source or in their processes of cell division and elongation (Herrera-Rodríguez *et al*., 2022; Hanc, Enev, Hrebeckova, Klucakova, and Pekar, 2019).

Table 2. Variability in fruit quality indicators of *Solanum lycopersicum* L., hybrid Caporal with doses of vermicompost, and inoculation with *S. rhizophila*.

Treatments	Firmness of the fruit	Acidity	Total soluble solids	Weight of the fruit
	N	%	°Brix	g
If	1.25±0.01 c	2.49±0.08 a	4.40±0.20 b	159.4±6.34 cd
Sr	1.27±0.05 c	1.96±0.03 b	4.64±0.27 b	165.8±16.57 c
V60	1.43±0.05 b	1.86±0.02 c	4.30±0.33 bc	141.4±14.17 d
V150	1.42±0.04 b	1.84±0.03 c	3.98±0.53 bc	163.6±14.38 c
V300	1.26±0.07 c	1.97±0.02 b	3.30±0.70 c	175.3±5.16 bc
Sr+V60	1.42 ±0.09 b	1.54±0.00 d	4.44±0.09 b	188.2±1.30 b
Sr+V150	1.53±0.02 ab	1.60±0.06 d	4.11±0.07 bc	194±6.47 b
Sr+V300	1.56±0.01 a	1.57±0.05 d	5.58±0.08 a	216±6 a
±EE	0.01	0.003	0.01	6.08
F	16	13.5	15	24.5
P		0.001		

Different superscripts in the same column suggest significant differences according to Tukey for $P \leq 0.05$.

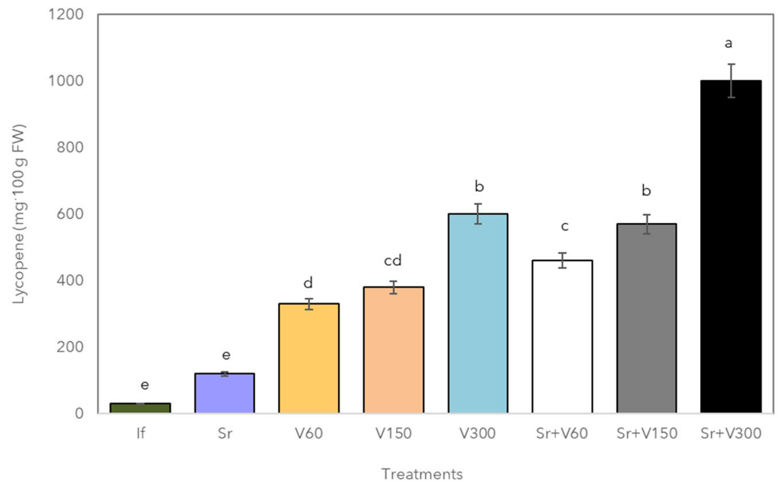

Figure 2. Effect of treatments on the amount of lycopene (mg 100 g fresh weight). Different superscripts represent significant differences, according to Tukey, for P ≤ 0.05. The vertical lines in the bars are the standard error (±).

The contents of P in the vermicompost used in this experiment are high (organic P 0.108% and total P 0.250%). In addition, *S. rhizophila* also produces indole-3-acetic acid (AIA), a plant hormone involved in cell elongation and division, tissue differentiation, and responses to light and gravity (Teheran-Sierra *et al*., 2021). In addition, the protection of plants from immunopathogens by this marine bacterium can positively influence plant growth and even more so when vermicompost is incorporated (Rivas-Garcia *et al*., 2019). Finally, bacterial of the genus *Stenotrophomonas* have a high capacity for adhesion and colonization of plant roots, which produces a more significant effect on their growth (Ulrich, Kube, Becker, Schneck, and Ulrich, 2021) and when interacting together with vermicompost stimulates its impact on plants. Diversity *Stenotrophomonas* species has been considered like a plant growth promoting rhizobacteria due to its ability to increase the morphology of various plants such as soybean (Egamberdieva, Jabborova, and Berg, 2016), wheat (Singh and Jha, 2017), chili (Kumar and Audipudi, 2015), tomato (Manh-Tuong *et al*., 2022), among others. In addition, vermicompost has increased various morphological parameters of crops such as tomatoes (Durukan, Demirbaş, and Tutar, 2019; Nava-Pérez, Valenzuela, and Rodríguez, 2019) and lettuce (Lee *et al*., 2020).

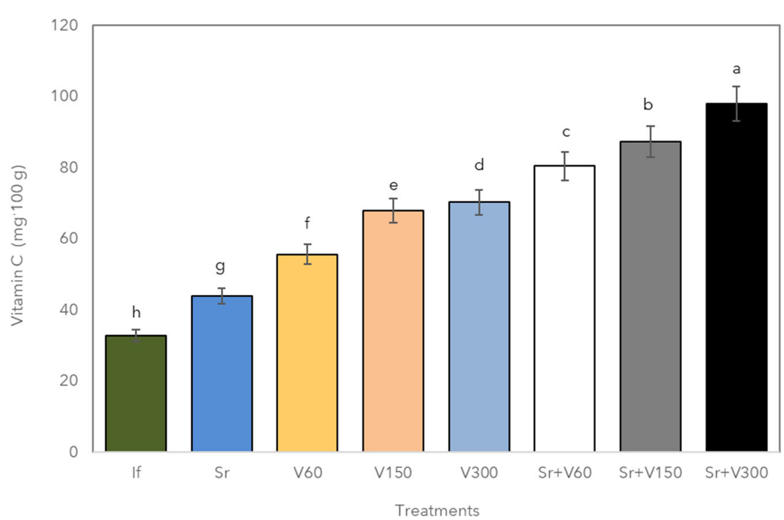

Figure 3. Effect of treatments on vitamin C in fruits (mg g in fresh weight). Different superscripts represent significant differences, according to Tukey, for *P* ≤ 0.05. The vertical lines in the bars are the standard error (±).

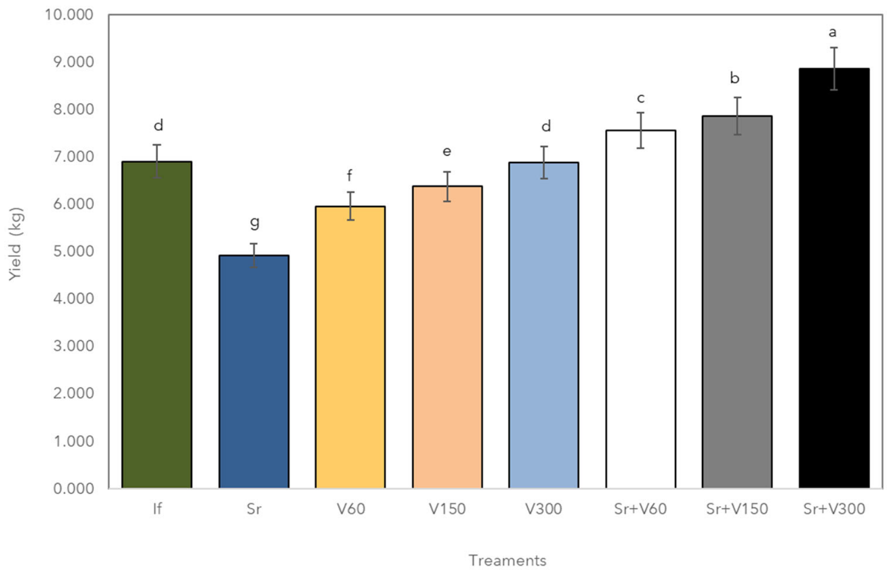

Figure 4. Effect of treatments on yield per plant (kg). Different superscripts represent significant differences, according to Tukey, for *P* ≤ 0.05. The vertical lines in the bars are the standard error (±).

The fruit quality parameters showed that the best treatment was *S. rhizophila* with 300 g of vermicompost (Sr+V300). These results are consistent with those reported by Espinosa-Palomeque *et al*. (2017) that the inoculation of rhizobacteria promoters of plant growth and the use of compost-based substrate increased the yield and nutraceutical quality of tomato fruits produced under greenhouse conditions. Similarly, these results agree with those reported by Lara-Capistrán *et al*. (2020b) in the cultivation of sweet chili from Yucatan by inoculating rhizobacteria promoters of plant growth and applying a dose of 570 g of vermicompost, had significant effects on this Solanaceae.

In °Brix, vitamin C, weight, and fruit yield the dose of 300 g of vermicompost with *S. rhizophila*, turned out to be the best. In this regard, Espinosa-Palomeque *et al*. (2017) mention that applying organic materials and incorporating bacteria that promote plant growth in tomato plants increased the number of total solids in the fruit. It is important to note that the fruits where the plants were fertilized presented the highest acidity values. This fact may be related to incorporating inorganic fertilizer that makes the fruits more acidic than those with a lower application (Terry-Alfonso, Ruiz, and Carrillo, 2018).

It is important to note that the values reported for °Brix were higher than those reported by Márquez-Hernández *et al*. (2013) for this same crop.

The same trend is observed for lycopene and vitamin C. It may be related to the presence of the bacterium since it has been seen in similar works reported by González-Rodríguez *et al*. (2018) that when using *Bacillus* sp., the content of this antioxidant was increased with an average of 5.65 mg 100 g^{-1} and if these results are compared with the one obtained this was higher than reported. Similarly, for vitamin C, if we compare with those reported by Terry-Alfonso, Ruiz, and Carrillo (2018) in this same crop with organic management with an average of 15.32 mg 100 g^{-1}.

Finally, regarding fruit weight and yield, it was observed that the high dose of vermicompost, in combination with *S. rhizophila* isolated from marine environments (Sr+V300) was the best. It can be attributed to the nutrients present in an environment such as vermicompost and the presence of *S. rhizophila* that makes these nutrients better assimilated by the plant and in greater concentration to be more bioavailable as observed in the present study, therefore helping growth and development, in a relatively short time, promoting an earlier start of fruit formation and production compared to only fertilized plants or even those in which only microorganisms were applied. This behavior coincides with what was established by González-Rodríguez *et al*. (2018), who indicate that when applying plant growth promoting rhizobacteria in tomato plants, they can increase yields due to the production of growth-stimulating substances. This fact suggests that the use of compost and the inoculation of bacteria are an option to increase the yield in this nightshade.

CONCLUSIONS

It is concluded that the use of these microorganisms isolated from marine environments in combination with vermicompost plant favors the antioxidant contents and yield of tomato fruits, being an alternative for use in these productive systems, which is desirable since during the last years they have received great interest for their antioxidant properties concerning free radicals, which suggests that these prevent the risks of acquiring chronic diseases such as cancer and cardiovascular diseases, also vermicompost together with *S. rhizophila* can reduce the use of inorganic fertilizer to 50% since it allows to obtain a higher yield and fruits of higher quality both for the national and international market. However, it would be necessary to make an economic analysis to consider vermicompost and biostimulants microorganisms of marine origin as viable products for cultivating tomatoes.

ETHICS STATEMENT

No applicable.

CONSENT FOR PUBLICATION

No applicable.

AVAILABILITY OF SUPPORTING DATA

The set of data used or analyzed in this research is available upon express request to the corresponding author.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FINANCING

No applicable.

AUTHORS' CONTRIBUTIONS

Conceptualization: L.L.C., and L.G.H.M. Methodology: L.L.C., A.G.R., and E.N.A.B. Formal analysis: B.M.A., and L.H.A. Research: L.L.C., E.N.A.B., and L.G.H.M. Resources: L.L.C. Writing: L.L.C., L.H.A., and L.G.H.M. Visualization: A.G.R., and B.M.A. Project Management: L.L.C.

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