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EVALUACIÓN DE LAS RESPUESTAS DE TRES VARIEDADES DE TOMATE (*Solanum lycopersicum* L.) INOCULADAS CON BACTERIAS CUANDO SE CULTIVAN EN CONDICIONES DE ESTRÉS POR AGUAS RESIDUALES Y SULFATO DE COBRE

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Evaluation of the responses of three tomato varieties (*Solanum lycopersicum* L.) inoculated with bacteria when grown under stress conditions due to wastewater and copper sulfate

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RESUMEN: El objetivo de esta investigación fue evaluar las respuestas de tres variedades *Solanum lycopersicum* L., inoculadas con cepas bacterianas, expuestas a condiciones de estrés por aguas residuales y sulfato de cobre, bajo condiciones de invernadero dentro de las instalaciones del Campus del Colegio de Postgrado Montecillo. Texcoco, México. El diseño experimental utilizado fue de bloques completamente al azar con un arreglo factorial, con tres repeticiones, dos niveles de sulfato de cobre, El sulfato es un compuesto que se encuentra en la naturaleza. Se encuentra de forma natural en el agua en diferentes cantidades. Si el agua contiene una gran cantidad de sulfato, puede tener un sabor amargo. Los sulfatos también se encuentran en minerales, suelo, rocas, plantas y alimentos. El sulfato de cobre pentahidratado ($CuSO_4·5H_2O$) y su uso en agricultura. Esto forma cristales grandes de color azul brillante que contienen cinco moléculas de agua ($CuSO_4·5H_2O$) y también se conoce como vitriolo azul. La sal anhidra se crea calentando el hidrato a 150 °C (300 °F). El sulfato cúprico se utiliza principalmente con fines agrícolas, como pesticida, germicida, aditivo para piensos y aditivo para el suelo.

Dos tipos de suelo regado con dos fuentes de riego (aguas residuales y aguas limpias), tres consorcios bacterianos, tres variedades de jitomate. *Lycopersicum* (dos mejoradas y una nativa de campesino) para comparar el efecto sobre el desarrollo de crecimiento fisiológico.

Se realizaron análisis de varianza y comparaciones de media (Tukey, $p \leq 0.05$). Mediante el uso de consorcios bacterianos se promovió el desarrollo del cultivado en condición de estrés por cobre y en suelos regados históricamente con aguas residuales durante 120 días. En la variedad R.G. 22 el consorcio bacteriano (1) de *P. putida, P. fluorescens* y A9 tuvo mayor influencia en el desarrollo de la planta, en la variedad R.G. 19, la mejor respuesta ocurrió con el uso del consorcio bacteriano 3 (A, D, A7), en la variedad R.N.

22 y el uso de los dos consorcios bacterianos (1 y 3) favoreció el desarrollo de las plántulas. Las evaluaciones de la respuesta de plántula en invernadero a la inoculación fueron similares, por lo que las dos evaluaciones permitieron comprobar que los consorcios bacterianos 1 y 3 tuvieron un efecto positivo en el desarrollo de las plántulas.

La variedad R.G. 22 mediante el uso del consorcio bacteriano 1 que incluye las bacterias *Pseudomonas putida, Pseudomonas fluorescens* y A9 presentó diferencias significativas con relación al tratamiento control. Se incrementó 11% la longitud del tallo y 3% el diámetro del tallo, se incrementó la longitud de la raíz en 14% y el volumen de la raíz en 33% y la biomasa seca de la parte aérea se incrementó en 14%, con respecto al testigo. En la variedad R.G. 19 mediante el uso del consorcio bacteriano 3 presentó un incremento 25% en la longitud del tallo y 7% de la raíz, 13% la biomasa seca de la parte aérea y 25% el área foliar. En la variedad R.N. 22 mediante el uso de los consorcios 1 y 3 se presentó un incremento en el número de hojas, 20% longitud y 60% biomasa de raíz y 17% en la biomasa seca de la parte aérea, en comparación con el testigo.

Palabras clave: presión hídrica, aguas residuales, biorremediación, consorcios bacterianos, variedades de jitomate,

ABSTRACT: The objective of this research was to evaluate the responses of three varieties of *Solanum lycopersicum* L., inoculated with bacterial strains and exposed to stress conditions by wastewater and copper sulfate under greenhouse conditions within the facilities of the Montecillo Graduate School Campus. Texcoco, Mexico. The experimental design used was completely randomized blocks with a factorial arrangement, with three replications and two levels of copper sulfate (with and without). Sulfate is a compound found in nature. It is found naturally in water in different quantities. The water may taste bitter if it contains a large amount of sulfate. Sulfates are also found in minerals, soil, rocks, plants, and foods. $CuSO_4·5H_2O$ and its use in agriculture. This forms large, bright blue crystals containing five water molecules ($CuSO_4·5H_2O$), also known as blue vitriol. Anhydrous salt is created by heating the hydrate to 300 °F (150 °C). Cupric sulfate is mainly used for agricultural purposes as a pesticide, germicide, feed additive, and soil additive. Two types of soil irrigated with two irrigation sources (wastewater and clean water), three bacterial consortia, and three varieties of tomato *lycopersicum* (two improved and one native peasant) were used to compare the effect on physiological growth development.

Analyses of variance and mean comparisons were performed (Tukey, $p \leq 0.05$). Using bacterial consortia, the crop's development was promoted under stress conditions by copper and in soils historically irrigated with wastewater for 120 days. In the R.G. 22 variety, the bacterial consortium (1) of *Pseudomonas putida, Pseudomonas fluorescens* and A9 had a more significant influence on the development of the plant, in the R.G. 19 variety, the best response occurred with the use of bacterial consortium 3 (A, D, A7), in the R.N. 22 variety and the use of the two bacterial consortia (1 and 3) favored the development of seedlings. The evaluations of the greenhouse seedling response to inoculation were similar, so the two evaluations allowed to verify that bacterial consortia 1 and 3 had a positive effect on seedling development. The R.G. 22 variety, through the use of bacterial consortium one that includes the bacteria *P. putida, P. fluorescens*, and A9, presented significant differences in the control treatment. The length of the stem and 3% diameter of the stem were increased, the length was increased by 14% and volume by 33% of the root, and the dry biomass of the aerial part increased by 14%, with respect to the control. In the R.G. 19 variety, through bacterial consortium 3, there was a 25% increase in stem length, 7% in root, 13% in the dry biomass of the aerial part, and 25% in leaf area. In the R.N. 22 variety, through the use of consortia 1 and 3, there was an increase in the number of leaves, 20% length and 60% root biomass, and 17% in the dry biomass of the aerial part, compared to the control.

Key words: water pressure, wastewater, bioremediation, bacterial consortia, tomato varieties.

INTRODUCTION

The global agricultural system is facing additional difficulties in the twenty-first century, including a drop in productivity and deterioration in the sustainability of agricultural ecosystems (Paice, 2022). Forecasts by the United Nations (United Nations, 2019) project that the global population will reach 9.7 billion people by 2050, resulting in a steady rise in food demand and its constrained available supply (Kumar *et al.*, 2017) Due to climate change, major cereal crops have seen significant production losses, with yield decreases of about 3.8% and 5% in reference to corn and wheat separately (Lipper *et al.*, 2014). Temperature rises have brought significant risks to agriculture, and the appearance of various abiotic factors has a negative impact on agricultural output (Paice, 2022). Many harmful abiotic environmental factors, such as salinity, turn arable land into barren land. This is because salinity drastically changes plant growth and metabolism, resulting in changes in plant physiology, morphology, and biochemistry (Gupta & Huang, 2014). By 2050, climate change will cause drought to impact over 50% of arable land (Osakabe *et al.*, 2014).

Drought affects crop hydration association, photosynthetic integration, and supplement utilization (Osakabe *et al.*, 2014). Heavy metal contamination is complex to remove and needed in small amounts for plant metabolic activities, but high levels harm the phytological and microbiological networks.

Food is necessary for survival and plays a crucial role in individual and societal development. Reduced crop yields are a significant cause for alarm. Pressure from a growing population on farmland to produce more food has resulted in the widespread usage of chemical inputs like fertilizers, herbicides, and insecticides. Through bioaccumulation and biomagnification, the effects of agrochemical runoff from such land have a negative impact on life on Earth. The use of insecticides to combat plant pests has unintended consequences for beneficial insects, soil fertility, and soil microbiota (Khatoon *et al.*, 2020). Harmful farming methods do not meet our needs and cannot provide a sustainable future. Sustainable agriculture has become increasingly important in recent times due to its focus on long-term environmental and social benefits, Knowing the importance of sustainable farming practices is crucial for meeting the world's future economic needs. These practices can help reduce the use of artificial pesticides and fertilizers while improving plant health and soil quality (de Andrade *et al.*, 2023)**.**

This soil quality is preserved with its diversity within sustainable agriculture, thus producing safe food for future generations, guaranteeing long-term environmental health. Sustainable agriculture must preserve the soil's inherent diversity to secure long-term environmental health worldwide and produce adequate food for future generations (Kumar *et al.*, 2017)**.**

Thus, eco-friendly alternatives, including the sustainable use of beneficial microorganisms, are crucial for alleviating environmental stress. Bacteria, known as plant growth-promoting rhizobacteria (PGPR), are the most important soil microbes. The benefits for the crop are achieved in many ways, such as nitrogen fixation, breaking down phosphate, getting rid of heavy metals, making phytohormones (like auxin, gibberellins, cytokinin, etc.), breaking down crop residue, and stopping phytopathogens from growing (He *et al.*, 2019)**.**

Researchers have demonstrated that PGPRs boost plant vitality and promote development without releasing harmful by-products into the atmosphere. With over 500 species, *Streptomyces* is the largest genus of Actinobacteria and a type genus of the Streptomycetaceae family among PGPRs (Mohammadipanah & Dehhaghi, 2017).

Crop production requires PGPR to boost plant nutrient availability since it directly or indirectly influences plant growth through root colonization (Hassanisaadi *et al.*, 2021). The PGPR enhances plant growth by bioremediating polluted soils by sequestering toxic heavy metal species and degrading xenobiotic compounds like pesticides. Additionally, it mobilizes soil nutrients, produces plant growth regulators, protects plants from phytopathogens by controlling or inhibiting them, and improves soil structure (Backer *et al.*, 2018).

Rhizosphere bacteria help cycle carbon through the earth and the environment and prevent soil carbon loss through metabolic activity. PGPRs can effectively control plant diseases brought on by fungi and bacteria. The phytomicrobiome, a group of beneficial bacteria, improves plant resilience to biotic and abiotic stress and agricultural productivity.

PGPR, a beneficial phytomicrobiome component, promotes plants' responses to biotic stress by producing chemical messengers, boosting their intake of nutrients, and releasing antibiotics (Lyu *et al.*, 2021).

In addition, one of the fundamental components of ecosystems is microbial communities that play important roles in the metabolism of organic matter and are involved in the degradation of a wide range of pollutants (Castillo Rogel *et al.*, 2020). This is why bacteria have been used to accumulate or metabolize different harmful compounds in the bioremediation process (Torres Rodríguez, 2003). Some species of bacteria with metabolic properties against harmful compounds are the following: *Pseudomonas putida, Deinococcus radiodurans, Cupriavidus metallidurans, Shewanella oneidensis*. Agriculture in Mexico is an important activity because of food production and because it generates jobs, foreign exchange, and relationships around the activity (Montaño Méndez *et al.*, 2021).

Fruit and vegetable activity represents 45% of exports, placing it as the most dynamic activity in terms of exports since the signing of the North American Free Trade Agreement, highlighting the export of products such as tomatoes (7.77%), peppers (3.9%), cucumbers (3.31%), lemons (2.86%), avocados (2.4%), and onions (2.23%) (Montaño Méndez *et al.*, 2021).

The tomato is one of the vegetables with the most significant economic importance worldwide. According to the Food and Agriculture Organization of the United Nations, Mexico ranks second in tomato exports, so increasing the production potential in adverse conditions is essential. The objective of this research was to evaluate the responses of three *Solanum lycopersicum* L. varieties inoculated with bacterial strains and exposed to stress conditions caused by wastewater and copper sulfate under greenhouse conditions.

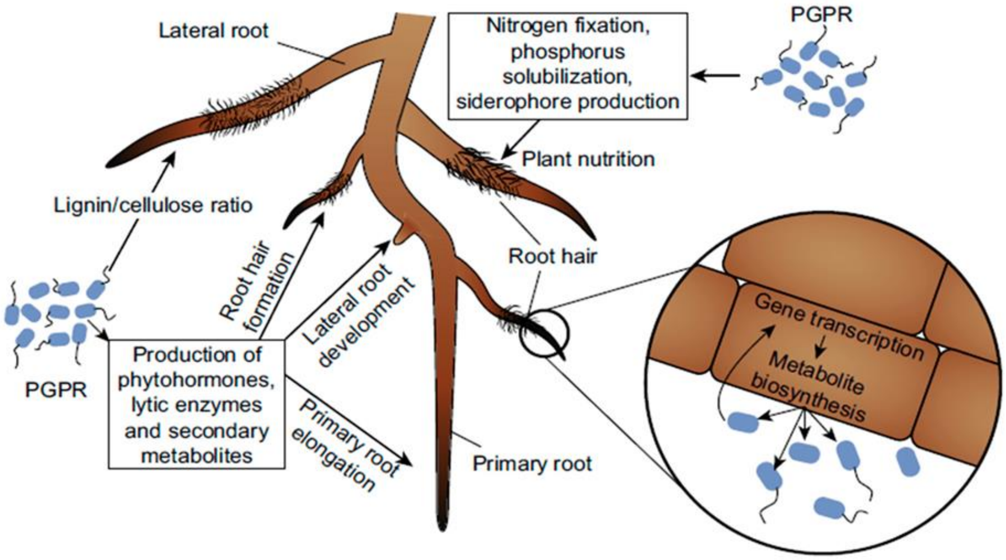

Figure 1. Impact of phytostimulating PGPR on RSA, nutrient acquisition and root functioning**.** PGPR can modulate root development and growth by producing phytohormones, secondary metabolites, and enzymes. The most commonly observed effects are a reduction of the growth rate of the primary root and an increase in the number and length of lateral roots and root hairs. PGPR also influences plant nutrition via nitrogen fixation, solubilization of phosphorus, or siderophore production, and modifies root physiology by changing gene transcription and metabolite biosynthesis in plant cells (Vacheron *et al.*, 2013) https://doi.org/10.3389/fpls.2013.00356

Figura 1. Impacto de la fitoestimulación con PGPR en la RSA, la adquisición de nutrientes y el funcionamiento radicular. La PGPR puede modular el desarrollo y el crecimiento radicular mediante la producción de fitohormonas, metabolitos secundarios y enzimas. Los efectos más comunes observados son la reducción de la tasa de crecimiento de la raíz primaria y el aumento del número y la longitud de las raíces laterales y los pelos radiculares. La PGPR también influye en la nutrición vegetal mediante la fijación de nitrógeno, la solubilización del fósforo o la producción de sideróforos, y modifica la fisiología radicular modificando la transcripción génica y la biosíntesis de metabolitos en las células vegetales (Vacheron *et al.*, 2013) https://doi.org/10.3389/fpls.2013.00356

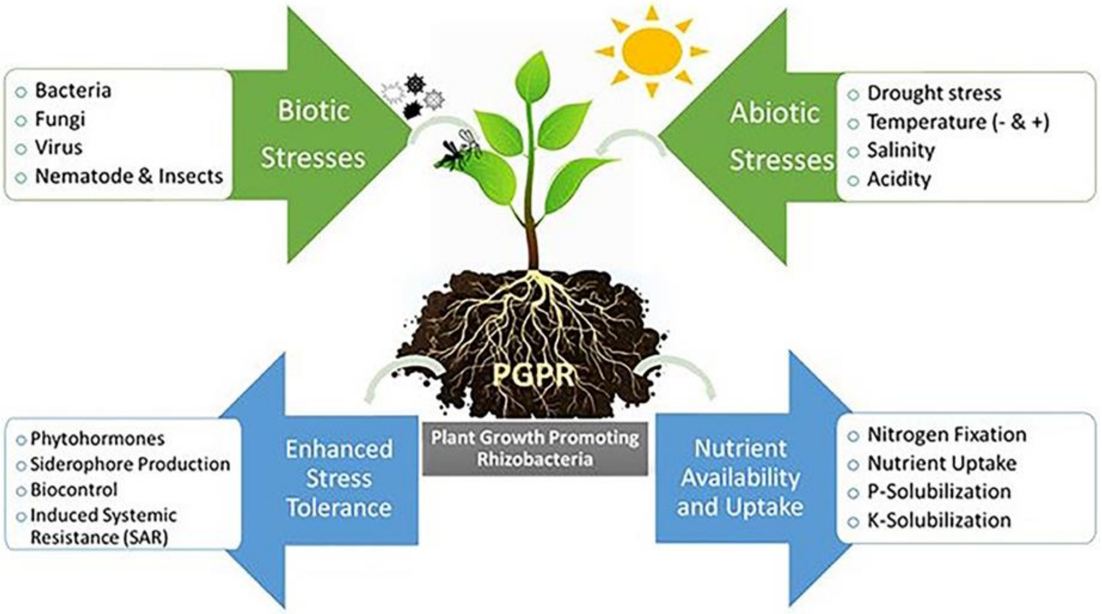

Figure 2. Plant growth promotes rhizobacteria (PGPR) and plant interactions in the rhizosphere (Vacheron *et al.*, 2013) https://doi.org/10.3389/fpls.2013.00356.

Figura 2. El crecimiento de las plantas promueve las rizobacterias (PGPR) y las interacciones de las plantas en la rizosfera (Vacheron *et al.*, 2013) https://doi.org/10.3389/fpls.2013.00356.

The application of beneficial microbes to food crops has been studied extensively; however, their implementation in the field is limited. Incorporating phytomicrobiome members in agricultural systems as a sustainable approach for disease management and nutrient supplements could reduce the adverse effects associated with the excess application of chemical inputs (fertilizers and pesticides) (Antar *et al.*, 2021). In addition, phytmicrobiome members have been employed as an effective strategy to mitigate certain biotic and abiotic stresses that could affect crop growth and production (Shah *et al.*, 2021).

METHODS

Experimental Site. The research was conducted under greenhouse conditions at the postgraduate college, Montecillo Campus, State of México at 19° 27´ N and 98° 54´ W. The Site has an altitude of 2245 meters above sea level, a mild climate, an average annual temperature of 16.4 °C, and a rainfall of 762.7 mm per year. The greenhouse had a flat, symmetrical triangular roof. The glass cover provided 70% light and was made of metal. Ventilation was provided through front and side windows. The laboratory tests took place in the Molecular Genetics Laboratory of the Postgraduate Program in Genetic Resources and Productivity of the Colegio de Postgraduados Campus Montecillo, Montecillo, Texcoco, Mexico. The greenhouse tests were carried out in a glass greenhouse within the facilities of the Colegio de Postgraduados Campus Montecillo, Montecillo, Texcoco, Mexico. The research was carried out in three stages; in the first, the viability of tomato seed exposed to $CuSO_4$ solutions, the effect of pre-germinative treatments as germination enhancers, and the response of the seed to inoculation with bacterial consortia were evaluated. The second stage consisted of determining the vigor of the tomato seedling whose seed was inoculated with bacterial consortia. In the third stage, the tomato plant was evaluated in a greenhouse up to 120 days after planting, developed under stress conditions by $CuSO_4$, and in soil irrigated with wastewater.

Biological material

Tomato seeds (*S. lycopersicum*) Rio Grande varieties (R.G. 22) had a purity of 99% and minimum germination of 90% in its last analysis in June 2022, Rio Grande variety (R.G. 19) purity of 99% with minimum germination of 92% in its last analysis in September 2019, and the native variety Kidney (R.N. 22), purity of 90% with germination of 95% in its last analysis in 2022, from Huatusco, Ver.

Table 1. Bacterial strains used to form bacterial consortia.
Tabla 1. Cepas bacterianas utilizadas para formar consorcios bacterianos

Bacterial strains	**Characteristic**
Pseudomonas. putida	bacteria fluorescence.
Pseudomonas fluorescens	bacteria fluorescence.
A9	Isolated from roots of corn crop bacteria, no fluorescence.
Avm	Isolated from roots of corn crop bacteria, no fluorescence.
7	Isolated from sludge from the Atlacomulco wastewater treatment plant bacteria fluorescence.
1	Isolated from sludge from the Atlacomulco wastewater treatment plant bacteria fluorescence.
A	Isolated from sludge from the Atlacomulco wastewater treatment plant bacteria fluorescence.
D	Isolated from sludge from the Atlacomulco wastewater treatment plant bacteria fluorescence.
A7	Isolated from roots of corn crop bacteria fluorescence.

Bacterial suspensions were prepared, for which cells (Figure 3) were transferred from each culture to test tubes containing distilled water. Suspensions adjusted to 0.9 (660 Nm) absorbance were formed in a spectrophotometer.

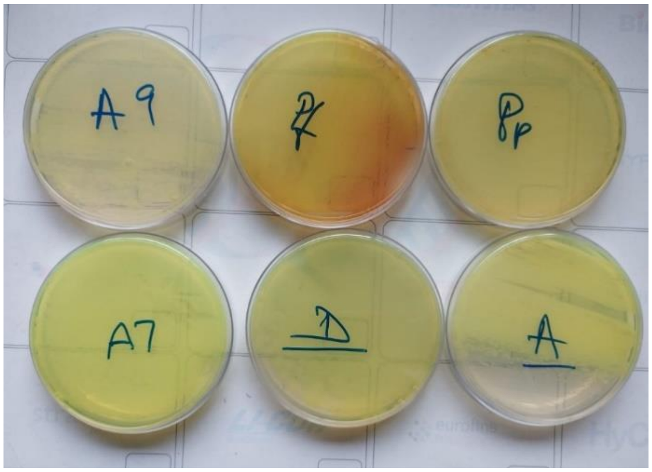

Figure 3. Bacterial strains. Pp (*Pseudomonas putida*), Pf (*Pseudomonas fluorescens*), A9 (Isolated from roots of corn crop bacteria no fluorescence), Avm (Isolated from roots of corn crop bacteria no fluorescence), 7 (Isolated from sludge from the Atlacomulco wastewater treatment plant bacteria fluorescence), 1 (Isolated from sludge from the Atlacomulco wastewater treatment plant bacteria fluorescence), A (Isolated from sludge from the Atlacomulco wastewater treatment

plant bacteria fluorescence), D (Isolated from sludge from the Atlacomulco wastewater treatment plant bacteria fluorescence), A7 (Isolated from roots of corn crop bacteria fluorescence).
Figura 3. Cepas bacterianas. Pp (*Pseudomonas putida*), Pf (*Pseudomonas fluorescens*), A9 (Aislada de raíces de cultivo de maíz, bacteria sin fluorescencia), Avm (Aislada de raíces de cultivo de maíz, bacteria sin fluorescencia), 7 (Aislada de lodos de la planta de tratamiento de aguas residuales de Atlacomulco, bacteria con fluorescencia), 1 (Aislada de lodos de la planta de tratamiento de aguas residuales de Atlacomulco, bacteria con fluorescencia), A (Aislada de lodos de la planta de tratamiento de aguas residuales de Atlacomulco, bacteria con fluorescencia), D (Aislada de lodos de la planta de tratamiento de aguas residuales de Atlacomulco, bacteria con fluorescencia), A7 (Aislada de raíces de cultivo de maíz, bacteria con fluorescencia).

Bacterial consortia. The Bacterial consortia were prepared by arranging groups of three bacteria whose growth is not inhibited by the presence of any other bacteria (Figure 4), as follows:

- Consortium 1: *Pseudomonas putida, Pseudomonas fluorescens* y A9
- Consortium 2: Avm, seven y 1
- Consortium 3: A, D, A7.

Figure 4. Growth of bacterial strains for consortia. Pp (*Pseudomonas putida*), Pf (*Pseudomonas fluorescens*), A9 (Isolated from roots of corn crop bacteria no fluorescence), Avm (Isolated from roots of corn crop bacteria no fluorescence), 7 (Isolated from sludge from the Atlacomulco wastewater treatment plant bacteria fluorescence), 1 (Isolated from sludge from the Atlacomulco wastewater treatment plant bacteria fluorescence), A (Isolated from sludge from the Atlacomulco wastewater treatment plant bacteria fluorescence), D (Isolated from sludge from the Atlacomulco wastewater treatment plant bacteria fluorescence), A7 (Isolated from roots of corn crop bacteria fluorescence).
Figura 4. Crecimiento de cepas bacterianas para consorcios. Pp (*Pseudomonas putida*), Pf (*Pseudomonas fluorescens*), A9 (Aislado de raíces de cultivo de maíz, bacteria sin fluorescencia), Avm (Aislado de raíces de cultivo de maíz, bacteria sin fluorescencia), 7 (Aislado de lodos de la planta de tratamiento de aguas residuales de Atlacomulco, bacteria con fluorescencia), 1 (Aislado de lodos de la planta de tratamiento de aguas residuales de Atlacomulco, bacteria con fluorescencia), A (Aislado de lodos de la planta de tratamiento de aguas residuales de Atlacomulco, bacteria con fluorescencia), D (Aislado de lodos de la planta de tratamiento de aguas residuales de Atlacomulco, bacteria con fluorescencia), A7 (Aislado de raíces de cultivo de maíz, bacteria con fluorescencia).

Of the prepared bacterial suspensions, equal volumes (0.9 ml) of suspensions were mixed from the bacteria that made up each consortium.

Soil

Two types of soil from the Mezquital Valley in the state of Hidalgo were used. The soil considered uncontaminated, which has historically been irrigated with spring (or clean) water, was extracted from El Manantial, Tezontepec de Aldama, Hidalgo.

Moreover, a second soil was used, brought from the locality Mixquiahuala de Juárez in the state of Hidalgo, which has historically been irrigated with wastewater.
Samples were obtained from the two types of soil at depths of 0-5 cm, 5-10 cm, and 10-40 cm. These samples were subjected to a soil analysis to determine the concentration of copper and its possible toxicity. Finally, for each soil type, a depth sample from 0-40 cm was used to establish the research study.

Solution of $CuSO_4$
The results obtained from the germination test in copper sulfate solution at different concentrations determined that the concentration to be used of $CuSO_4$ pentahydrate is 10-2 M. This concentration had effects on the germination process, but these were moderate.

RESULTS

The variable days to flowering only presented statistically significant differences in the variety R.G. 22 in the soil type factor ($p \leq 0.05$), increasing 9.13% the time flowering occurred in the soil historically irrigated with wastewater in relation to the soil irrigated with clean water. Although statistically, this variable did not vary in the soil type of the R.G. 19 variety and in the R.N. 22 variety, the flowering time also increased (Table 1).

Figure 5. Flower of tomato cultivation
Figura 5. Flor del cultivo del tomate.

Table 2. Days to flowering by factor and level.
Tabla 2. Días a floración según factor y nivel.

Factors	**Variety**		
	R.G. 22	R.G. 19	R.N. 22
Soil type			
Soil irrigated with clean water	93.67 a	98.17 a	82.28 a
Soil irrigated with wastewater	102.22 b	103.28 a	79.33 a
Stress condition			
Without $CuSO_4$	100.28 a	102.83 a	78.67 a
With $CuSO_4$	95.61 a	98.61 a	82.94 a
Bacterial consortium			
Control	101.92 a	99.33 a	82.58 a
1	93.17 a	105.50 a	78.33 a
3	98.75 a	97.33 a	81.50

Note: Averages with the same letter per column do not differ statistically (Tukey, $p \leq 0.05$).

Consortium 1: *Pseudomonas putida, Pseudomonas fluorescens* y A9, Consortium 3: A, D, A7.

The leaf area's magnitude defines the vegetation cover's ability to intercept photosynthetically active radiation (PAR), which is the primary source of energy used by plants to manufacture tissues and produce food compounds (Warnock *et al.*, 2006).

Leaf area. It is an important variable in most agricultural and physiological studies involved in plant growth, light harvesting, photosynthetic efficiency, respiration, and transpiration. Leaf area determination is generally performed by direct methods, in which leaves taken from plants are analyzed with the help of an integrated electronic area meter (Casierra Posada Peña Z G. R. & Peña Olmos J. E., 2007).

The leaf area was evaluated for a young leaf, which has reached its maximum development per plant of each treatment per variety at the end of the trial, i.e., on day 120 after planting. In the R.G 22 variety, the soil type factor influenced the leaf area since it differed statistically ($p \leq 0.05$) between the soil irrigated with clean water and the soil irrigated with wastewater (Table 3). Likewise, the bacterial consortium factor had significant differences between inoculation and control treatments.

The variety R.G.19 presented statistical differences ($p \leq 0.05$) in the factors of soil type and bacterial consortium. The leaf area was lower at the soil level, irrigated with residual, and at the control level, the treatment in which the seed was not inoculated.

The leaf area of the R.N. 22 variety had significant differences ($p \leq 0.05$) in the stress condition factor, reducing in exposure to $CuSO_4$. In addition, the bacterial consortium was statistically different from the control treatment.

Table 3. Leaf area (cm^2) by factor and level.
Tabla 3. Área foliar (cm^2) por factor y nivel.

Factors and levels	**Variety**		
	R.G. 22	R.G. 19	R.N. 22
Soil type			
Soil irrigated with clean water	103.46 b	100.76 b	35.40 a
Soil irrigated with wastewater	68.06 a	72.69 a	34.71 a
Stress condition			
Without $CuSO_4$	86.57 a	85.70 a	37.74 b
With $CuSO_4$	84.95 a	87.75 a	32.36 a

Bacterial consortium			
Control	73.17 a	74.25 a	30.22 a
1	86.41 ab	92.45 b	36.25 ab
3	97.71 b	93.48 b	38.69 b

Note: Averages with the same letter per column do not differ statistically (Tukey, $p \leq 0.05$).

The leaf area is an indicator of photosynthetic efficiency since several processes are carried out in plants, such as leaf senescence, fruit ripening, internode elongation, dormancy, closure and opening of stomata, etc., which are regulated by phytohormones (auxins, gibberellins, cytokinins, abscisic acid, and ethylene) (Daza-Martínez *et al.*, 2018).

In the varieties R.G. 22 and R.N. 22, the bacterial consortium 3 was the one that presented a significant increase with respect to the control; in the variety R.G. 19, the two consortia had a positive effect, observing an increase in the leaf area.

Root length

The root length evaluated at 120 days after planting in the variety R.G. 22 differed statistically ($P \leq 0.05$) in the soil type factor (Table 4), reducing in the soil irrigated with wastewater, in the same way, in the bacterial consortium factor consortium 1, which includes the *P. putida* bacteria, *P. fluorescens*, and A9 showed significant differences in relation to the control treatment.

The root is the bottom of the plant's shaft and is usually buried in the soil, although roots develop in the air or water. A plant's set of roots in the soil is called the root system. The roots' main functions are fixing the plant to the soil and absorbing water and mineral salts. Other functions are storage, the synthesis of plant hormones, the aeration of the plant in aquatic environments, as a means of propagation of new plants, etc. In many species, the root is symbiontly associated with certain species of fungi to form mycorrhizae, and some plants, such as legumes, can also associate with bacteria in a symbiotic way, forming structures called nodules (Megías *et al.*, 2020).

On the other hand, in the variety R.G. 19, there were significant differences ($p \leq 0.05$) for the bacterial consortium factor, where consortium 3, made up of bacteria A, D, and A7, had a longer root length than the control.

In the case of the R.N. 22 variety, the statistical difference ($p \leq 0.05$) was presented for the bacterial consortium factor between the control and the two bacterial consortia used.

Table 4. Root length (cm) by factor and level.
Tabla 4. Longitud de raíz (cm) por factor y nivel.

Factors and levels	**Variety**		
	R.G. 22	R.G. 19	R.N. 22
Soil type			
Soil irrigated with clean water	39.44 b	36.61 a	33.67 a
Soil irrigated with wastewater	34.94 a	36.44 a	32.50 a
Stress condition			
Without $CuSO_4$	37.11 a	37.78 a	33.39 a
With $CuSO_4$	37.28 a	35.28 a	32.78 a
Bacterial consortium			
Control	33.67 a	32.33 a	29.00 a
1	41.58 b	37.83 ab	35.17 b
3	36.33 ab	39.42 b	35.08 b

Note: a) Control treatment, b) Inoculation treatment with consortium 1, c) Inoculation treatment with consortium 3.

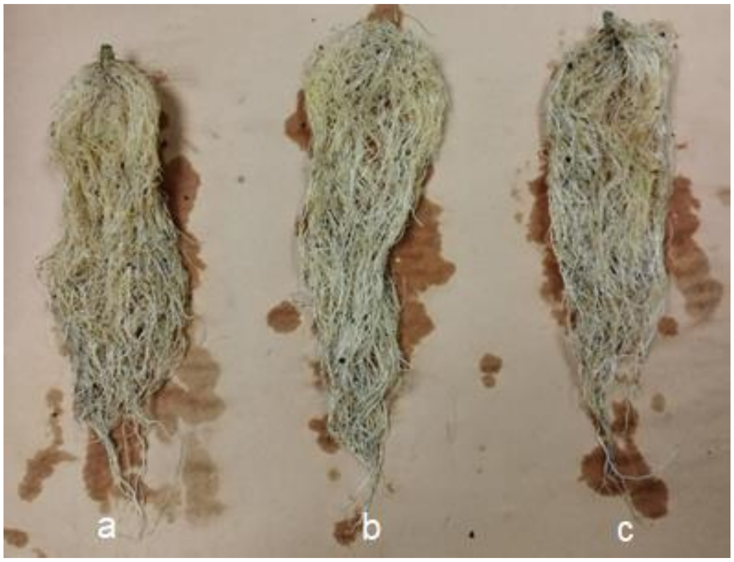

Figure 6. Tomato plant root.
Figura 6. Raíz de la planta de tomate.

A stage essential for plant growth-promoting bacteria to promote plant development is the colonization of the root system. Root colonization includes four stages: attraction, recognition, adhesion, and invasion, all of which are affected by biotic and abiotic factors. Microorganisms that settle on seeds during germination can grow and colonize the roots to their full extent. Seed colonization during the impregnation or immersion phase significantly affects plant growth (Moreno Reséndez *et al.*, 2018).
In this study, in the R.G. 22 variety, the results obtained with the bacterial consortium 1 differ statistically from the control; in the R.G. 19 variety, the bacterial consortium 3 had a positive effect and differed from the control; in the R.N 22 variety, a favorable response was presented with the use of the two consortia. Similarly, (Lyu *et al.*, 2021) point out that root length has a greater development by inoculating with plant growth-promoting bacteria in tomato cultivation under greenhouse conditions.

Root volume
Considering the soil type factor, the three varieties had significant differences ($P \leq 0.05$) in the root volume variable (Table 5), reducing considerably in the soil historically irrigated with wastewater. On the contrary, no statistically significant differences existed in the stress condition factor. Regarding the bacterial consortium factor, in the R.G. 22 variety, the root volume differed statistically between the control treatment and the inoculation treatment with the number 1 consortium.

Table 5. Root volume (cm^3) by factor and level.
Tabla 5. Volumen radicular (cm3) según factor y nivel.

Factors and levels	**Variety**		
	R.G. 22	R.G. 19	R.N. 22
Soil type			
Soil irrigated with clean water	30.28 b	28.61 b	22.50 b
Soil irrigated with wastewater	23.33 a	23.06 a	14.17 a
Stress condition			
Without $CuSO_4$	25.83 a	26.39 a	18.61 a
With $CuSO_4$	27.78 a	25.28 a	18.06 a
Bacterial consortium			
Control	22.08 a	25.83 a	17.50 a
1	30.42 b	23.75 a	18.33 a
3	27.92 ab	27.92 a	19.17 a

Note: Averages with the same letter per column do not differ statistically (Tukey, $p \leq 0.05$).

Bacteria can be important plant allies since some, in interaction, produce various metabolites that stimulate or inhibit metabolic pathways that favor plant growth. In tomato cultivation, the plants benefit from their interaction with these bacteria~~.~~
Likewise, (Daza-Martínez *et al.*, 2018) indicates that the use of growth-promoting bacteria in tomato cultivation stimulates the development of the root system, presenting a greater volume, which coincides with the results obtained in the R.G. 22 Variety-

DISCUSSION

With the increasing prominence of environmental issues, reducing the use of chemical synthetic fertilizers and pesticides have become a consensus among people (Keeler, B.L., 2016). Microbial interactions in the rhizosphere with plant growth-promoting microorganisms can act as a desired approach to enhancing plant productivity, promoting litter decomposition and soil fertility, and accelerating nutrient cycling (Rodríguez-Caballero *et al.*, 2018). In many plants, such as potato (*Solanum tuberosum*), tomato (*Lycopersicum esculentum*), coliflor (*Arabidopsis thaliana*), and maize (*Zea mays*), it has been shown that microbial combinations have a greater potential to promote plant growth compared to the application of single bacterial strains (Rolli *et al.*, 2015; Rivett *et al.*, 2018).

Days to flowering

Days to flowering. The flower is the reproductive organ of most plants. The spermatophyte group, i.e., seeded plants, is represented by gymnosperms and angiosperms. Both groups of plants have flowers, but in the case of gymnosperms, the flowers are inflorescences that will not give rise to a fruit. On the contrary, angiosperms have typical flowers that, after fertilization, will form seeds enclosed in fruits (Megías *et al.*, 2020). Flowers appear during the plants' reproductive season through a process called flowering. Like all plant organs, flowers originate from meristematic activity. The apical meristem transforms size, organization, and mitotic activity and becomes an apex for reproduction (Megías *et al.*, 2020). (Morales Castillo H., 2009) indicates that tomato varieties of certain growth begin between 55 and 60 days after planting, while those of indeterminate growth begin between 65 and 75 days after planting.
The variable days to flowering only presented statistically significant differences in the variety R.G. 22 in the soil type factor ($P \leq 0.05$), increasing 9.13% the time flowering occurred in the soil historically irrigated with wastewater in relation to the soil irrigated with clean water.

Although statistically, this variable did not vary in the soil type of the R.G. 19 variety and in the R.N. 22 variety, the flowering time also increased. Bacterial consortia did not influence the days of flowering in the tomato crop, which counted from planting. In the results obtained by (Carrillo Castaneda et al., 2000), there were significant differences in the number of days to flowering of plants inoculated with bacterial strains; however, according to the type of strain used, this difference was positive generating precocity, or negative prolonging the flowering process, compared to the control.

Bacterial consortia did not influence the days of flowering in the tomato crop, which counted from planting. In the results obtained by (Carrillo Castaneda *et al.*, 2000), there were significant differences in the number of days to flowering of plants inoculated with bacterial strains; however, according to the type of strain used, this difference was positive generating precocity, or negative prolonging the flowering process, compared to the control.

Leaf area

The leaf area's magnitude defines the vegetation cover's ability to intercept photosynthetically active radiation (PAR), which is the primary source of energy used by plants to manufacture tissues and produce food compounds (Warnock *et al.*, 2006). Leaf area is an important variable in most agricultural and physiological studies involved in plant growth, light harvesting, photosynthetic efficiency, respiration, and transpiration. Leaf area determination is generally performed by direct methods, in which leaves taken from plants are analyzed with the help of an integrated electronic area meter (Casierra Posada Peña Z G. R. & Peña Olmos J. E., 2007). The leaf area was evaluated for a young leaf, which reached its maximum development per plant of each treatment per variety at the end of the trial, i.e., on day 120 after planting, the leaf area increased by 25% with respect to the control treatment. In the R.G 22 variety, the soil type factor influenced the leaf area since it differed statistically ($p \leq 0.05$) between the soil irrigated with clean water and irrigated with wastewater. Likewise, the bacterial consortium factor had significant differences between inoculation and control treatments.

The variety R.G.19 presented statistical differences ($p \leq 0.05$) in soil type and bacterial consortium factors. The leaf area was lower at the soil level irrigated with residual and at the control level, the treatment in which the seed was not inoculated. The leaf area of the Rn 22 variety had significant differences ($p \leq 0.05$) in the stress condition factor, reducing exposure to $CuSO_4$. In addition, the bacterial consortium was statistically different from the control treatment.

The leaf area is an indicator of photosynthetic efficiency since several processes are carried out in plants, such as leaf senescence, fruit ripening, internode elongation, dormancy, closure, and opening of stomata, which are regulated by phytohormones (auxins, gibberellins, cytokinins, abscisic acid, and ethylene) (Daza-Martínez *et al.*, 2018). In their research (Daza-Martínez *et al.*, 2018) pointed out that the leaf area of the tomato crop through the use of bacteria increases with respect to the control, which coincides with the results obtained. In the varieties R.G. 22 and the bacterial consortium 3 was the one that presented a significant increase (25%) with respect to the control; in the variety R.G. 19, the two consortia had a positive effect, observing an increase in the leaf area.

Root Length

The root is the lower part of the plant's shaft and is usually buried in the ground, although roots develop in the air or in water. A plant's set of roots in the soil is called the root system. The roots' main functions are fixing the plant to the soil and absorbing water and mineral salts. Other functions are storage, the synthesis of plant hormones, the aeration of the plant in aquatic environments, and the propagation of new plants. In many species, the root is associated in a symbiotic way with certain species of fungi to form mycorrhizae. Also, some plants, such as legumes, can associate with bacteria in a symbiotic way, forming structures called nodules (Megías *et al.*, 2020).

The root length evaluated at 120 days after planting in the variety R.G. 22 differed statistically ($p \leq 0.05$) in the soil type factor, reducing in the soil irrigated with wastewater, in the same way, in the bacterial consortium factor consortium 1, which includes the *P. putida* bacteria, *P. fluorescens*, and A9 showed significant differences in relation to the control treatment.

On the other hand, in the variety R.G. 19, there were significant differences ($p \leq 0.05$) for the bacterial consortium factor, where consortium 3, made up of bacteria A, D, and A7, had a longer root length than the control.

In the case of the R.N. 22 variety, the statistical difference ($p \leq 0.05$) was presented for the bacterial consortium factor between the control and the two bacterial consortia used. A stage essential for plant growth-promoting bacteria to promote plant development is the colonization of their root system. Root colonization includes four stages: attraction, recognition, adhesion, and invasion, all affected by biotic and abiotic factors. Microorganisms that settle on seeds during germination can grow and colonize the roots to their full extent. Seed colonization during the impregnation or immersion phase significantly affects plant growth (Moreno Reséndez *et al.*, 2018).

In this study, in the R.G. 22 variety, the results obtained with the bacterial consortium 1 differ statistically from the control; in the R.G. 19 variety, the bacterial consortium 3 had a positive effect and differed from the control; in the Rn 22 variety, a favorable response was presented with the use of the two consortia. Similarly (Sánchez López D. B., Garrido Rubiano M. F. & Bonilla Buitrago R. R, 2018) point out that root length has a greater development by inoculating with plant growth-promoting bacteria in tomato cultivation under greenhouse conditions.

Root volume Considering the soil type factor, the three varieties had significant differences ($p \leq 0.05$) in the root volume variable (Table 4), reducing considerably in the soil historically irrigated with wastewater. On the contrary, no statistically significant differences existed in the stress condition factor. Regarding the bacterial consortium factor, in the R.G. 22 variety, the root volume differed statistically between the control treatment and the inoculation treatment with the number one consortium.

Bacteria can be important plant allies since some, in interaction, produce various metabolites that stimulate or inhibit metabolic pathways that favor plant growth. In tomato cultivation, the plants benefit from their interaction with these bacteria. The root volume of the inoculated plants was greater than that of the non-inoculated plants, improving their ability to explore and enabling them to assimilate nutrients better (Andrade-Sifuentes *et al.*, 2022).

Likewise, (Daza-Martínez *et al.*, 2018) indicates that the use of growth-promoting bacteria in tomato cultivation stimulates the development of the root system, presenting a greater volume, which coincides with the results obtained in the R.G. 22 variety.

CONCLUSIONS

Factors such as temperature, seed hydration, and seed storage time determined the germination process. In addition, pre-germination hydration treatments allowed seeds to recover their germination capacity with a long storage time.

The evaluations of the seedling response in vitro and the greenhouse to inoculation were similar, so the two evaluations allowed to verify that bacterial consortia 1 and 3 had a positive effect on seedling development. Using bacterial consortia, the development of tomatoes grown under copper stress conditions and in soils historically irrigated with wastewater for 120 days was promoted. In the R.G. 22 variety, bacterial consortium 1 (P. putida, P. fluorescens, and A9) had a greater influence on plant development; in the R.G. 19 variety, the best response occurred with the use of bacterial consortium 3 (A, D, A7) and in the Rn 22 variety, the use of the two bacterial consortia (1 and 3) favored plant development.

In this way, bacterial consortia 1 and 3 promoted the development of the tomato plant grown under copper stress conditions and in soils irrigated with wastewater. However, differences between varieties were presented. The R.G. 22 variety, through the use of bacterial consortium one that includes the bacteria *P. putida*, *P. fluorescens*, and A9, presented significant differences in relation to the control treatment. The length of the stem and 3% diameter of the stem were increased, the length was increased by 14% and volume by 33% of the root, and the dry biomass of the aerial part increased by 14%, with respect to the control. In the R.G. 19 variety, through the use of bacterial consortium 3, there was a 25% increase in stem length, 7% in root, 13% in the dry biomass of the aerial part, and 25% in leaf area. In the R.N. 22 variety, through the use of consortia 1 and 3, there was an increase in the number of leaves, 20% length and 60% root biomass, and 17% in the dry biomass of the aerial part, compared to the control.

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