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An Ecological Strategy to Reduce and Optimize Nitrogenous Fertilizer in the Growth of *Solaneum lycopersicum*

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Abstract: *Solanum lycopersicum* (tomato) is one of the most common agricultural crops in the world. Its production in Mexico and the world depends on the application of nitrogenous fertilizer such as NH_4NO_3, which, used rationally or in reduced doses according to the needs of the plant, avoids environmental pollution in addition to the emission of N2O (oxide nitrous), greenhouse gas. One option to reduce and optimize is to apply *Azotobacter vinelandii* and *Xanthobacter autotrophicus* with a crude extract of carbon nanoparticles (CENC). The objective of this research was to analyze the effect of *A. vinelandii* and *X. autotrophicus* on the growth of *S. lycopersicum* at 70% NH_4NO_3plus a CENC. The experiment was carried out under a randomized block experimental design with two controls, six treatments and six replications. The response variables were: days of emergence and percentage (%) of germination, phenology: plant height (PH) and root length (LR) and biomass: aerial and root fresh and dry weight: (AFW/RFW) / (ADW/ RDW) at the seedling and flowering stages; experimental data was analyzed by ANOVA/Tukey ($P<0.01$). The results showed a positive effect of *A. vinelandii* and *X. autotrophicus* at 70% NH_4NO_3 and CENC on the germination percentage of *S. lycopersicum* seeds, as well as on phenology and biomass. In the seedling and flowering stages, the numerical values showed a statistical difference compared to *S. lycopersicum* without *A. vinelandii and X. autotrophicus*, or CENC, only at 100% NH_4NO_3 (relative control). These results support that S. lycopersicum fertilized at 50% NH_4NO_3 inoculated with *A. vinelandii and X. autotrophicus* was optimized and improved by CENC to prevent soil fertility loss and possible N_2O emission.

Keywords: Soil, Nitrogen Fertilizer, Plant Growth Promoting Bacteria, Nanoparticules, Greenhouse Effect Gases

Introduction

The healthy growth of plants of agricultural importance such as *Solanum lycopersicum* (tomato) requires the application of nitrogenous fertilizer such as NH_4NO_3. One of the main problems in current agriculture is that part of the nitrogenous fertilizer is not adequately uptake by the roots of the plant, which can cause the soil as a non-renewable resource to be lost, while the nitrogenous fertilizer is converted in nature into a greenhouse gas such as N_2O or a contaminant of surface or groundwater (Isbell et al., 2013; Soto- Mora et al., 2016; Studdert et al., 2017; Silva et al., 2017). An ecological solution strategy is to inoculate the seeds of *S. lycopersicum* with *Azotobacter vinelandii* in a mixture with *Xanthobacter autotrophicus*, which, being plant endophytes, can improve the radical uptake of NH_4NO_3 despite reducing it to 70% of the recommended dose so that it is optimized to the maximum. specifically if it is combined with a crude extract of carbon nanoparticles (CENC) (Hatami, 2017; Chaudrhary et al., 2021; Merinero et al., 2022) that facilitates the rapid and maximum uptake of 70% NH_4NO_3 not leaving any remnant of nitrogenous fertilizer that decreases productivity from the soil and avoid as much as possible the release of N_2O, a greenhouse gas. According to the literature, there is little information related to agricultural importance. The objective of this research was to analyze the growth of *S. lycopersicum* inoculated with *A. vinelandii* and *X. autotrophicus* at 70% NH_4NO_3 and a CENC.

Material and methods

This research was carried out in the greenhouse of the Environmental Microbiology Laboratory of the Biological Chemical Research Institute of the UMSNH, Morelia, Mich, Mexico. The average temperature was 23.2 °C and the relative humidity was 67%. A non-sterile agricultural soil was selected from a site located at 19º 41' 23.5" north latitude 101º 15' 00.5" west longitude, with an altitude of 1920 meters above sea level from the facilities of the Natural Resources Research Institute from Morelia, Michoacan, Mexico. The texture of this soil was classified as loam according to NOM-021.RECNAT-2000 (Table 1), which was sieved with a mesh and solarized to reduce pests and diseases. Subsequently 1.0 kg of soil was placed in the upper part of a Leonard jar (Figure 1), while water or NH_4NO_3 in a mineral solution in the lower part, both parts were connected by a cotton strip to allow movement. of the liquid by capillarity to the ground (Sanchez-Yañez, 2007)

The *A. vinelandii* and *X. autotrophicus* strains belong to the collection of the Environmental Microbiology Laboratory of the Chemical Biological Research Institute of the UMSNH. A. vinelandii was activated in Burk agar and *X. autotrophicus* in agar without

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nitrate and sucrose (AWNS), incubated at 36°C/30-35 h, then the colony-forming colonies (CFU)/mL were counted. for both bacteria (Sánchez-Yáñez, 2007). While the CENC was obtained from leaves of *Albizia* sp, they collected Ciudad Universitaria, UMSNH, Morelia, Mich., which were disinfected with NaCl, rinsed with sterile deionized H_2O, then cut into pieces, dried at 80 °C/12 h, of which 30 g suspended in 300 mL of deionized H_2O were used, immediately heated to 70 °C/30 min, then filtered in Whatman No. 1, centrifuged at 4000 rpm/10 min and it was refrigerated. The partial characterization of the ECNC was carried out using a JEOL JSM-6400 scanning electron microscope from the Institute for Research in Metallurgy and Materials of the Research in Metallurgy and Materials of the UMSNH, Morelia, Mich, Mexico. To determine the qualitative and quantitative composition of the ECNC, it was carried out by means of dispersive energy (EDS), for which a JEOL-JSM-7600F EDS field emission microscope was used.

In plastic bags for every 10 seeds of , *S. lycopersicum* with 1.0 ml of *A. vinelandii and/or X. autotrophicus* in a 1:1 (v/v) ratio, then treated with 10 and 20 ppm of the CENC , were shaken at 200 rpm/30 min at 28°C, then planted in the soil of Leonard's jars according to the experimental design in Table 2 with two controls, six treatments and six replications in *S. lycopersicum:* uninoculated irrigated with water as an absolute control (AC); uninoculated, fed with 100% NH_4NO_3 or relative control (RC); with *A. vinelandii and X. autotrophicus* at 70% NH_4NO_3 enhanced with a CENC. NH_4NO_3 was applied every 3 days for three months in a mineral solution containing the following chemical composition (g/L): NH_4NO_3 12.0, KH_2PO_4 3.0, K_2HPO_4 3.5, $MgSO_4$ 1.5, $CaCl_2$ 0.1, $FeSO_4$ 0.5 ml and 1.5 ml of the solution of trace elements (g/L): H_3BO_3 2.86, $ZnSO_4$*7H_2O 0.22, $MnCl_2$*7 H_2O 1.81, K_2MnO_4 0.09 in 1000 ml of distilled water; with pH adjusted to 6.8. The response variables used were: germination percentage, phenology: plant height (PH) and root length (RL); and biomass: aerial and root fresh weight (AFW/RFW) and aerial and root dry weight (ADW/RDW) in seedling at 30 days and flowering at 60 days stages after sowing (Sánchez-Yáñez, 2007). The experimental data obtained were subjected to analysis of variance (ANOVA) using the Tukey HSD comparative test of means ($P<0.01$) with the statgraphics centurión statistical program.

To demonstrate that healthy growth of *S. lycopersicum* was due to the beneficial effect of *A. vinelandii* and *X. autotrophicus on,* the Kirby-Bauer technique was used to obtain the antibiotic sensitivity profile of *A. vinelandii* and *X. autotrophicus*. before and after inoculating the seeds of *S. lycopersicum* The sensitivity profile was for Gram-negative bacteria genera and species: ampicillin (AM), cefotaxime (CF), ceftazidime (CTX), cefuroxime (CAZ), pefloxacin (CXM), tetracycline (DC), and trimethoprim-sulfamethoxazole (E). , cephalothin (GE), dicloxacin (PEF), erythromycin (PE), gentamicin (TE), and penicillin (STX).

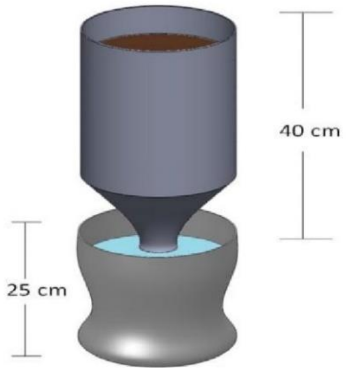

Figure 1. Leonard's jar design

Table 1 Physicochemical proprieties of agricultural soil

Parameters*	Value
pH (1:2)	6.68
Electrical conductivity: 2 (H_2O) (ms/cm)	0.33
Apparent density (s/mL)	0.80
Organic matter (%)	2.27
Texture	Loam
Soil bulk density (g/cm^3)	0.92
Total, nitrogen (%)	0.15
Nitric nitrogen (ppm)	30.16
Potassium (ppm)	368
Phosphorus (ppm)	4.65

*Physicochemical parameters for agricultural soils according to NOM-021-RECNAT-2000.

Table 1 shows the physicochemical properties of the soil prior to the test, which registered a slightly acid pH that conditions the solubility of PO_4^{-3}, with a medium organic matter content that indicates a poor imbalance in the C: N; with a clay texture. These factors condition the healthy growth of, *S. lycopersicum* (Contreras-Santos et al., 2020).

Figure 2 shows the partial characterization of the CENC by SEM micrograph, showing the morphology and sizes of the carbon nanomaterials present in the crude extract synthesized from *Albizia* sp leaves. As a result, spherical shapes lower than the 200 nm dispersed and forming cluster (Figure 2a). The EDS analysis recorded some chemical elements that could be involved in the formation of nanoparticles, of which carbon predominated with 50.98 atomic%, followed by oxygen (O_2) with 30.41 atomic% and a remainder of magnesium (Mg), phosphorus (P), chlorine (Cl) and potassium (K) with 18.6 Atomic % (Figure 2b) (Abdelmoteleb et al., 2017;).

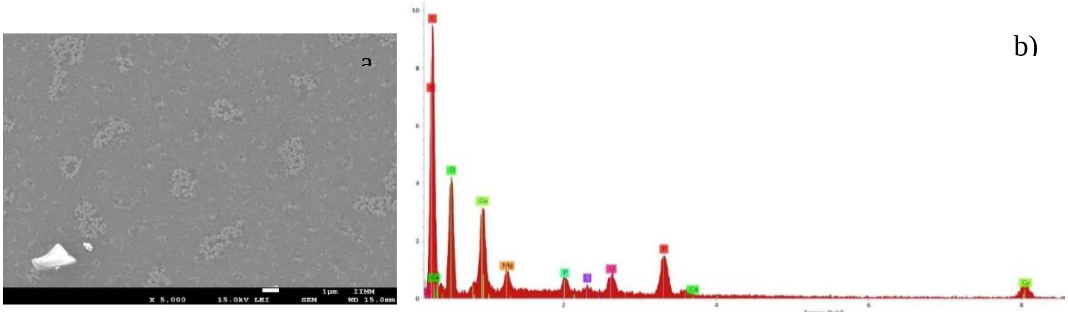

Figure 2. Partial characterization of the crude extract of carbon nanoparticles: a) scanning electron microscopy micrograph and b) graph of the scattered energy spectrum.

Table 2 shows that seeds of *S. lycopersicum* inoculated with *A. vinelandii* plus *X. autotrophicus* at 70% NH_4NO_3 and 10/20 ppm of CENC, registered 93% germination. This results was due to that the seeds release organic metabolites by the action of starch hydrolysis, which *A. vinelandii* and *X. autotrophicus* transformed into phytohormones that were improved with an CENC, increased the percentage of germination of *S. lycopersicum* (Lira-Saldivar et al., 2018; Vásconez et al., 2020), as shown in figure 3, had numerical value was statistically different compared to 70.5% germination of *S lycopersicum* without *A. vinalandii/X. autotrophicus*, fed at 100% NH_4NO_3, without CENC or relative control (RC).

Table 2. Effect of *Azotobacter vinelandii* and *Xanthobacter autotrophicus* on germination of *Solanum lycopersicum* at 70% NH_4NO_3 enhanced with a CENC.

Solanum lycopersicum*	**Percentage of germination (%)**
Irrigated with water or absolute control (AC)	$56.3\%^{d**}$
Fed with 100% NH_4NO_3 or relative control (RC)	$70.5\%^{c}$
A. vinelandii + 70% NH_4NO_3 + 10 ppm CENC	$87.1\%^{b}$
X. autotrophicus + 70%NH_4NO_3 + 10 ppm CENC	$86.7\%^{b}$
A. vinelandii and *X. autotrophicus* + 70%NH_4NO_3 + 10 ppm CENC	$93\%^{a}$
A. vinelandii + 70% NH_4NO_3 + 20 ppm CENC	$83.9\%^{bc}$
X. autotrophicus + 70% NH_4NO_3 + 20 ppm CENC	$86.4\%^{b}$
A. vinelandii and *X. autotrophicus* + 70% NH_4NO_3 + 20 ppm CENC	$85.3\%^{b}$

*n=6, **values with different letters had statistical difference (P<0.01) according to ANOVA/Tukey.

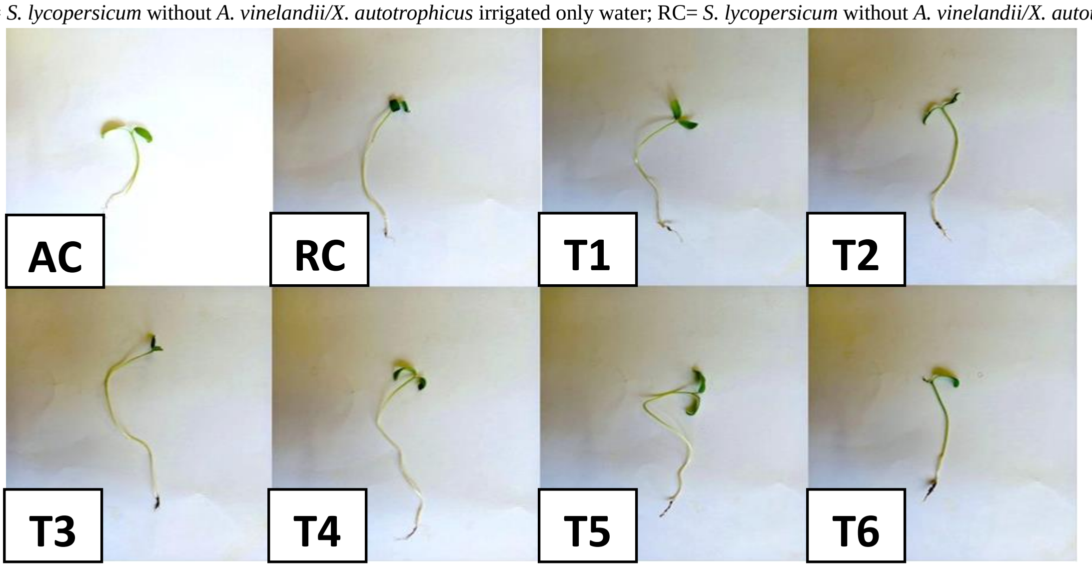

Figure 3. Effect of *Azotobacter. vinelandii* and *Xanthobacter autotrophicus* on the germination of *S. lycopersicum* at 70% NH_4NO_3 enhanced with a CENC at 7 days after sowing

AC= *S. lycopersicum* without *A. vinelandii/X. autotrophicus* irrigated only water; RC= *S. lycopersicum* without *A. vinelandii/X. autotrophicus*, fed at 100% NH_4NO_3 untreated with CENC: T1= *S. lycopersicum* + A. *vinelandii* + a70% NH_4NO_3 + 10 ppm CENC; T2= *S. lycopersicum* + *X. autotrophicus* + 70% NH_4NO_3 + 10 ppm CENC; T3= *S. lycopersicum* + *A. vinelandii/X. autotrophicus* + 70% NH_4NO_3 + 10 ppm CENC; T4= *S. lycopersicum* + *A. vinelandii* + 70% NH_4NO_3 + 20 ppm CENC; T5= *S. lycopersicum* + *X. autotrophicus* + 70% NH_4NO_3 + 20 ppm CENC; T6= *S. lycopersicum* + *A. vinelandii/X. autotrophicus* + 70% NH_4NO_3 + 20 ppm CENC.

Table 3 shows the phenology and fresh and dry biomass of *S. lycopersicum* at the seedling level inoculated with *A. vinelandii* and *X. autotrophicus* at 70% NH_4NO_3 enhanced with 20 ppm of CENC, where it registered 0.07 g of DAW and 0.06 g of DRW. Numerical values with statistical difference comparad to the 0.04 g of DAW and the 0.04 g of RDW of *S. lycopersicum* fed only with 100% NH_4NO_3. The results supports that *X. autotrophicus* was able to invaded the interior of the roots *S. lycopersicum* in order to converted carbon compounds derived from photosynthesis into phytohormones that maximized radical uptake of 70% NH_4NO_3 an specific physiological plant activity which was enhancing with a CENC (Ahmed et al., 2021).While in the figure 4 is show the response of *S. lycopersicum* at the seedling level inoculated with *A. vinelandii* and *X. autotrophicus* at 70% NH_4NO_3 enhanced with 20 ppm of CENC.

Table 3. Response of *S. lycopersicum* at seedling to *A. vinelandii* and *X. autotrophicus* at 70% NH_4NO_3 enhanced with a CENC.

Solanum lycopersicum*	**Plant height (cm)**	**Root length (cm)**	**Fresh weight (g)**		**Dry weight (g)**	
			Aerial	**Radical**	**Aerial**	**Radical**
Irrigated with water or absolute control (AC)	15^{b**}	2.5^{c}	0.41^{cd}	0.12^{d}	0.02^{c}	0.02^{c}
Fed with 100% NH_4NO_3 or relative control (RC)	15.7^{b}	5.2^{b}	0.53^{c}	0.16^{c}	0.04^{b}	0.04^{bc}
A. vinelandii + 70% NH_4NO_3 + 10 ppm CENC	8.5^{d}	5^{b}	0.48^{c}	0.19^{ab}	0.06^{ab}	0.04^{bc}
X. autotrophicus + 70%NH_4NO_3 + 10 ppm CENC	7.5^{d}	5.75^{a}	0.56^{c}	0.21^{a}	0.05^{b}	0.09^{a}
A. vinelandii and *X. autotrophicus* + 70%NH_4NO_3 + 10 ppm CENC	11^{cd}	6.5^{a}	0.44^{b}	0.22^{a}	0.06^{a}	0.08^{a}
A. vinelandii + 70% NH_4NO_3 + 20 ppm CENC	13.5^{c}	4.8^{b}	0.77^{b}	0.19^{ab}	0.06^{a}	0.05^{b}
X. autotrophicus + 70% NH_4NO_3 + 20 ppm CENC	17.5^{a}	6.5^{a}	0.86^{ab}	0.23^{a}	0.06^{a}	0.04^{bc}
A. vinelandii and *X. autotrophicus* + 70% NH_4NO_3 + 20 ppm CENC	15.5^{b}	5.5^{ab}	0.93^{a}	0.18^{b}	0.07^{a}	0.06^{b}

*n=6, **values with different letters had statistical difference ($P<0.01$) according to ANOVA/Tukey.

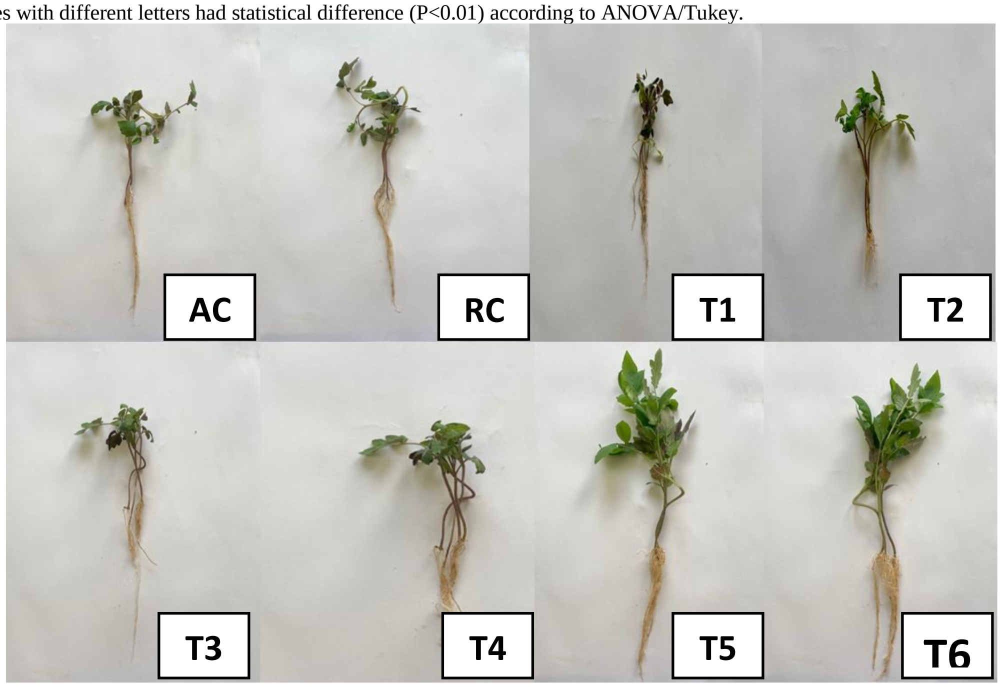

Figure 4. Effect of *A. vinelandii* and *X. autotrophicus* on phenology of *S. lycopersicum* at seedling level at 70% NH_4NO_3 enhanced with a CENC.

AC= *S. lycopersicum* without *A. vinelandii/X. autotrophicus* irrigated only water; RC= *S. lycopersicum* without *A. vinelandii/X. autotrophicus*, fed at 100% NH_4NO_3 untreated with CENC: T1= *S. lycopersicum* + A. *vinelandii* + 70% NH_4NO_3 + 10 ppm CENC; T2= *S. lycopersicum* + *X. autotrophicus* + 70% NH_4NO_3 + 10 ppm CENC; T3= *S. lycopersicum* + *A. vinelandii/X. autotrophicus* + 70% NH_4NO_3 + 10 ppm CENC; T4= *S. lycopersicum* + *A. vinelandii* + 70% NH_4NO_3 + 20 ppm CENC; T5= *S. lycopersicum* + *X. autotrophicus* + 70% NH_4NO_3 + 20 ppm CENC; T6= *S. lycopersicum* + *A. vinelandii/X. autotrophicus* + 70% NH_4NO_3 + 20 ppm CENC.

Table 4 shows the phenology, as well as the fresh and dry biomass of *S. lycopersicum* at flowering inoculated with *A. vinelandii* and *X. autotrophicus* at 70% NH_4NO_3 plus 20 ppm of CENC. In that sense was registered 0.013 g of DAW and 0.025 g of DRW, numerical values with statistical difference compared to 0.08 g DAW and 0.008 g DRW of *S. lycopersicum* used as relative control. According to the literature and the data registered on the phenology and biomass of *S. lycopersicum* with *A. vinelandii* and *X. autotrophicus* plus the CENC, accelerated and improving the uptake of NF at 70% due to better radical system (Khodakovskaya et al., 2009), as is showed on Figure 5.

Table 4. Effect of *A. vinelandii* and *X. autotrophicus* on phenology and biomass of *S. lycopersicum* at flowering at 70% NH_4NO_3 enhanced with a CENC

Solanum lycopersicum*	**Plant height (cm)**	**Rood length (cm)**	**Fresh weight (g)**		**Dry weight (g)**	
			Aerial	**Radical**	**Aéreo**	**Radical**
Irrigated with water or absolute control (AC)	21.5c**	3.8c	0.72c	0.21d	0.06b	0.04d
Fed with 100% NH_4NO_3 or relative control (RC)	23.7b	6.2b	0.85b	0.29c	0.08b	0.08d
A. vinelandii + 70% NH_4NO_3 + 10 ppm CENC	28.5a	7.8a	0.82b	0.35a	0.012a	0.08d
X. autotrophicus + 70%NH_4NO_3 + 10 ppm CENC	27.5a	8.7a	0.87b	0.3b	0.012a	0.014c
A. vinelandii and *X. autotrophicus* + 70%NH_4NO_3 + 10 ppm CENC	22b	7.5a	0.75bc	0.35a	0.011a	0.021ab
A. vinelandii + 70% NH_4NO_3 + 20 ppm CENC	23.8b	6.9b	0.81b	0.29c	0.012a	0.024a
X. autotrophicus + 70% NH_4NO_3 + 20 ppm CENC	26.1a	7.8a	1.33a	0.33a	0.014a	0.021ab
A. vinelandii and *X. autotrophicus* + 70% NH_4NO_3 + 20 ppm CENC	25.9ab	7.7a	1.4a	0.32ab	0.013a	0.025a

*n=6, **values with different letters had statistical difference (P<0.01) according to ANOVA/Tukey.

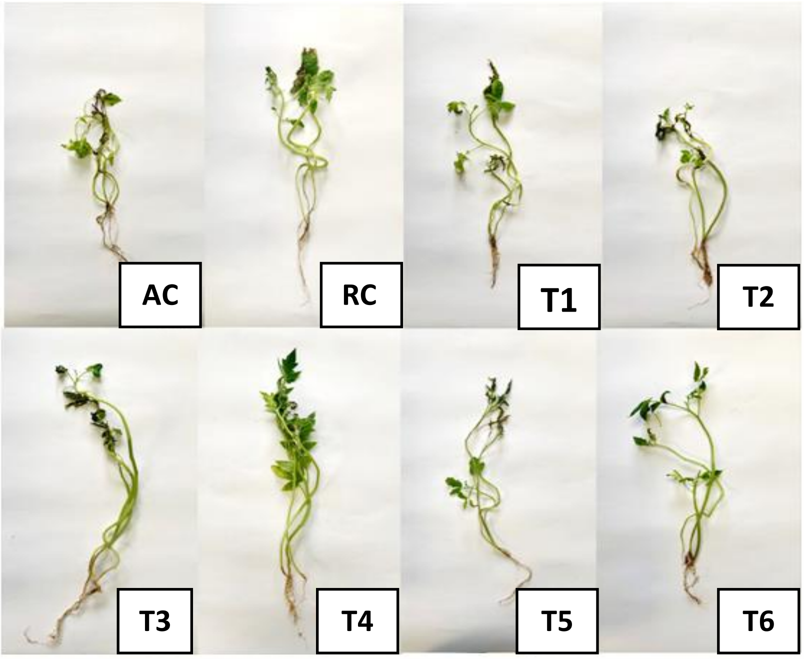

Figure 5. Effect of *Azotobacter vinelandii* and *Xanthobacter autotrophicus* on *Solanum. lycopersicum* at flowering at 70% NH_4NO_3 enhanced with a CENC.

AC= *S. lycopersicum* without *A. vinelandii/X. autotrophicus* irrigated only water; RC= *S. lycopersicum* without *A. vinelandii/X. autotrophicus*, fed with 100% NH_4NO_3 untreated with CENC: T1= *S. lycopersicum* + A. *vinelandii* + 70% NH_4NO_3 + 10 ppm CENC; T2= *S. lycopersicum* + *X. autotrophicus* + 70% NH_4NO_3 + 10 ppm CENC; T3= *S. lycopersicum* + *A. vinelandii/X. autotrophicus* + 70% NH_4NO_3 + 10 ppm CENC; T4= *S. lycopersicum* + *A. vinelandii* + 70% NH_4NO_3 + 20 ppm CENC; T5= *S. lycopersicum* + *X. autotrophicus* + 70% NH_4NO_3 + 20 ppm CENC; T6= *S. lycopersicum* + *A. vinelandii/X. autotrophicus* + 70% NH_4NO_3 + 20 ppm CENC.

Table 5 shows the antibiogram of *A. vinelandii* and *X. autotrophicus* endophytes used as reference strains to demonstrate the beneficial effect on the phenology and biomass of *S. lycopersicum* at seedling and flowering stages. In that sense *A. vinelandii* and *X. autotrophicus* it has being recovered from the root, stem, and leaves of the several domestic plants. While according to the method of Kirby and Bauer, registered the profile of antibiotic sensitivity: CF, CTX, CAZ, GE, PEF, PE, TE and resistant to CXM, DC, E and SXT. What supports *A. vinelandii* and *X. autotrophicus* invaded the xylem of *S. lycopersicum* for healthy growth of *S. lycopersicum* (Merinero et al., 2022). This sensitivity profile was compared and similar to that obtained by *A. vinelandii and X. autotrophicus* before and after recovery from domestic plants fed only with 100% NH_4NO_3, while neither of the two bacterial genera and species was isolated from them. plants used as RC.

Table 6. Antibiogram of *A. vinelandii* and *X. autotrophicus* recovered from *S. lycopersicum*

Antibiotics	Reference strain		*A. vinelandii* y *X. autotrophicus* recovered from *S. lycopersicum*		
	Azotobacter vinelandii	*Xanthobacter autotrophicus*	Leaf	Stem	Root
Ampicillim (AM)	-	-	-	-	-
Cefotaxime (CF)	-	-	-	-	-
Ceftazidime (CTX)	-	-	-	-	-
Cefuroxime (CAZ)	-	-	-	-	-
Pefloxacin (CXM)	+	+	+	+	+
Tetracycline (DC)	+	+	+	+	+
Trimethoprim-sulfamethoxazole (E)	+	+	+	+	+
Cephalothin (GE)	-	-	-	-	-
Dicloxacin (PEF)	-	-	-	-	-
Erythromycin (PE)	-	-	-	-	-
Gentamicin (TE)	-	-	-	-	-
Penicillin (STX)	+	+	+	+	+

(+) = sensitive; (-) = resistant.

Conclusion

The positive response of *S. lycopersicum to A. vinelandii and X. autotrophicus* at 70% NH_4NO_3 with the CENC supports that its healthy growth was due to the action of bacterial phytohormones that optimized the uptake of NH_4NO_3 at a reduced dose enhanced and accelerated by the CENC. In this sense, nitrogen fertilization of the soil can be used intelligently to avoid the loss of soil productivity, environmental pollution, as well as the generation of N_2O, a greenhouse gas.

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Conflicts of interest

The participants in this research assure that there is no problem of interests related to the planning, execution and reporting of this research that compromises the value of the results obtained or their consequences in scientific, technical, or any other type of terms.

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