Research Article

Growth of *Cucumis sativus, Phaseolus vulgaris and Solanum lycopersicum with Azotobacter vinelandii and Xanthobacter autotrophicus* at 50% NH_4NO_3 plus crude carbon nanoparticle extract reduces N_2O release in soil

Abstract

The healthy growth of *Phaseolus vulgaris, Cucumis sativa and Solanum lycopersicum* requires nitrogen fertilizer such as NH_4NO_3, that when is applied in doses higher than the actual uptake causes loss of soil fertility, as well as the release of N_2O, due to soil physicochemical conditions that inducing a greenhouse gas that contributes to global warming. The objetives of this research were: i) to analyze the response of *P. vulgaris, C. sativa* and *S. lycopersicum* to 50% NH_4NO_3 with *Azotobacter vinelandii* and *Xanthobacter autotrophicus,* plus crude carbon nanoparticles extract (CCNPE), ii) to determine the effect of *A. vinelandii* and *X. autotrophicus* on the yield elements of *P. vulgaris* with 50% NH_4NO_3 and a CCNPE. For this aims, seeds of *P. vulgaris, C. sativa and S. lycopersicum* were inoculated with *A. vinelandii and X. autotrophicus* with 50% NH_4NO_3 and a CCNPE, with the response variables being percentage and days to germination, phenology and biomass at seedling and pre-flowering, and, in the case of *P. vulgaris*, yield elements. Experimental data were analyzed using ANOVA-Tukey. The results showed that *P. vuglaris, C. sativa* and *S. lycopersicum* with *A. vinelandii* and *X.autotrophicus* with 50% NH_4NO_3 plus CCNPE: reduced germination time of all seeds, increased germination percentage plant height, root length, fresh and dry weight of aerial and radical since both genera of plant growth promoting endophytic bacteria, when colonizing the seeds and roots of these plants converted plant metabolism compounds into phytohormones to increase the uptake of 50% NH_4NO_3, an action accelerated by the CCNPE with statistically different numerical values compared to the same seeds with 100% NH_4NO_3 uninoculated neither CCNPE. It is concluded that mixing of *A. vinelandii and X. autotrophicus* optimized the maximum 50% of NH_4NO_3, especially with the CCNPE, to prevent NO_3 remaining from non-uptake by the root system of the plants, that could be converting to N_2O under the physical and chemical conditions of the soil, to avoid the release this N_2O and prevent global warming.

Keywords: soil, domestic plants NH_4NO_3, beneficial plant endophytes, agricultural N_2O mitigation climate

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Introduction

The healthy growth of *Phaseolus vulgaris* (beans) *Cucumis sativus* (cucumber), and *Solanum lycopersicum* (tomato) require nitrogen fertilizer such as NH_4NO_3.[1–3] When applied in excess, this fertilizer causes rapid mineralization of soil organic matter, with a consequent decrease in agricultural productivity, besides the releasing of N_2O according to microbial activity in this type of soil causing global warming.[4,5] An alternative ecological solution that avoids nitrogen overfertilization and to prevent N_2O releasing is to inoculate seeds of *C. sativus, P. vulgaris* and *S. lycopersicum* with *Azotobacter vinelandii* and/or *Xanthobacter autotrophicus* genera and endophytic bacterial species, that promote growth through the synthesis of phytohormones and a regulated dose of NH_4NO_3.[6,7] In the literature, it is reported that *A. vinelandii* and *X. autotrophicus* shorten the days to emergence, with an increase in the germination percentage,[8,9] as well as optimize the uptake of NH_4NO_3 to 50% for healthy plant growth by phytohormonal induction of a root system with higher density (10). Like *X. autotrophicus,* as an endophyte, it can convert from the root products of the metabolism of these plants, into phytohormones that allow healthy plant growth.[7,11] It is possible that *A. vinelandii* and *X. autotrophicus* inoculated individually or in combination in seeds of *C. sativus, P. vulgaris* and *S. lycopersicum* ensure healthy growth with a dose of 50% NH_4NO_3.[5,11] One option is a crude carbon nanoparticles extract (CCNPE or CCNP), that enhances the phytohormonal activity of both endophytic plant growth promoting bacteria.[8,12,13] Since according to some authors,[1–3] report that *Cicer arietinum* and *T. aestivum* with a regulated dose of nitrogen fertilizer and CCNPE, that had a positive response in germination and healthy growth.[1,3,5] The objetives of this research were: i) to analyze the response of *C. sativa, P. vulgaris* and *S. lycopersicum* to 50% NH_4NO_3 with *A. vinelandii* and *X. autotrophicus* plus CCNPE, ii) to determine effect of *A. vinelandii* and *X. autotrophicus* on the yield elements of *P. vulgaris* with 50% NH_4NO_3 and a CCNPE that to optimize dose of 50% NH_4NO_3 and to prevent N_2O releasing that mitigate global warming.

Material and methods

This research was conducted in the Environmental Microbiology Laboratory of the Chemical-Biological Research Institute (CHBRI) of the UMSNH, Morelia, Michoacán, México. The soil was solarized to reduce pests and diseases; it was subsequently sieved with a No.

20 mesh screen; the field capacity was determined at 80%, equivalent to 280 mL/kg, to allow for water and oxygen exchange.[1,14] Setup of a semi-hydroponic system or Leonard jar. 1 kg of soil was weighed into the upper part of the semi-hydroponic system known as a Leonard jar, and water or a 100% or 50% mineral solution was added to the lower part, as it is shown in Figure 1. Origin of *A. vinelandii and X. autotrophicus* were taken from the collection of the Environmental Microbiology Laboratory of the CHBRI of the UMSNH. *A. vinelandii* and *X. autotrophicus* were activated and two culture media were prepared. *A. vinelandii* was reproduced in Burk agar with the following chemical composition (g/L): Glucose 10.0, KH_2PO_4 2.0, K_2HPO_4 2.0, $MgSO_4$ 3.0, Yeast Extract 1.0, Bacteriological Agar 18.0 Trace Elements Sol. 1 mL, Bromothymol Blue 10 mL, Tecto® 10 mL, distilled water 1000 mL, the pH was adjusted to 7.8. Meanwhile, *X. autotrophicus* was grown on Nutrient Agar with the following chemical composition (g/L): glucose 10.0, peptone 5.0, yeast extract 1.0, bacteriological agar 18.0. Both culture media were incubated at 30°C/24-36 h.[1,15] The density of the viable inoculum was determined from 1.0 mL of *A. vinelandii* and/or *X. autotrophicus* previously suspended in 0.85% detergent saline solution (SSD), shaken for 30 min, and diluted in test tubes with 9 mL of 0.85% saline solution and detergent to a 10^{-8} dilution. From the 10^{-2}, 10^{-4} and 10^{-6} dilutions, 0.1 mL was taken and inoculated in triplicate in the center of the Petri dish. It was spread with the Driglaski loop and the dishes were inverted, for *A. vinelandii* in Burk agar and for *X. autotrophicus* in NSNA.[1,16] The dishes with the culture medium were incubated at 30 ° C / 24-36 h. From the expected growth of *A. vinelandii* in Burk agar and *X. autotrophicus* in Nutrient agar, the colony forming units (CFU) / mL were counted. For *A. vinelandii* it was 1.17 X 10^9 CFU / mL and for *X. autotrophicus* it was 1.59 X 10^6 CFU / mL as it is shown in Figure 1. Figure 2 Macroscopic (a) and microscopic (b) morphology of *Azotobacter vinelandii* on Burk agar. Figure 3 Macroscopic (a) and microscopic (b) morphology of *Xanthobacter autotrophicus* in nutrient agar.

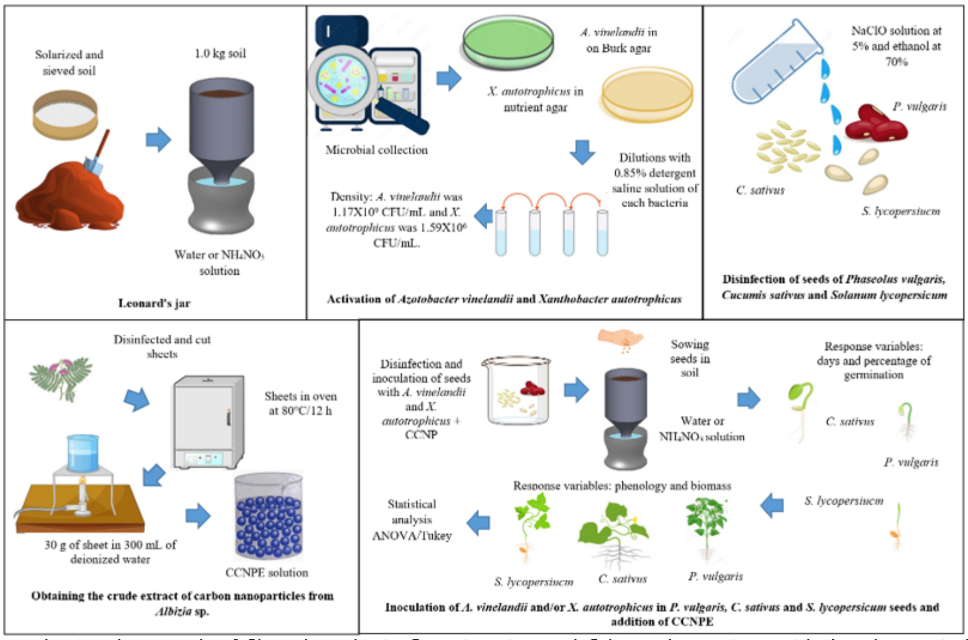

Figure 1 Diagram for evaluating the growth of *Phaseolus vulgaris, Cucumis sativus* and *Solanum lycopersicum* with *Azotobacter vinelandii* and *Xanthobacter autotrophicus* at 50% NH_4NO_3 plus crude carbon nanoparticle extract reduces N_2O release in soil.

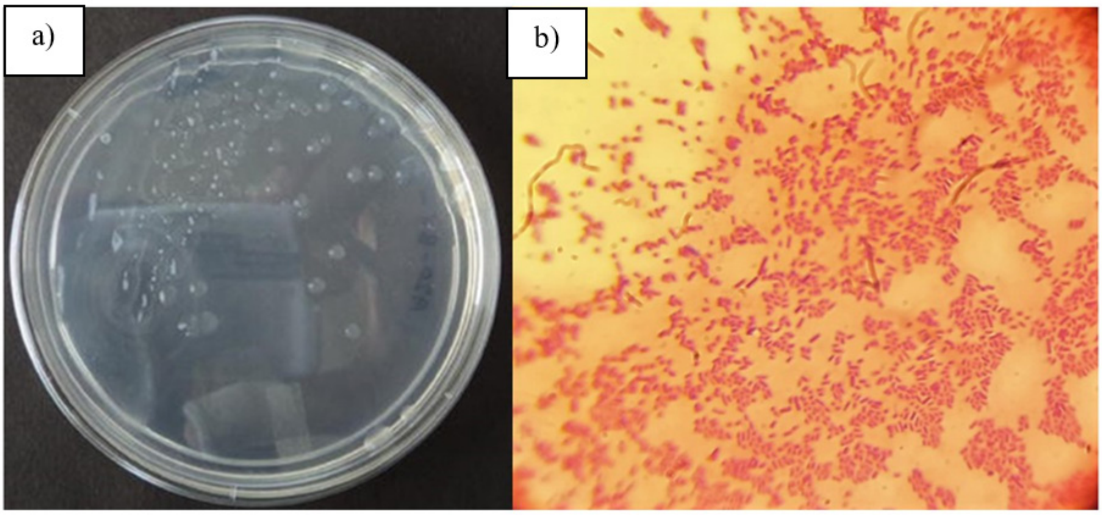

Figure 2 (a) shows creamy white colonies and (b) varying in morphology from rods to coccus-shaped cells. *X. autotrophicus* are observed as individual cells, in pairs, or forming irregular aggregates, and sometimes forming chains of variable size shows large, short, Gram-negative by Gram staining.

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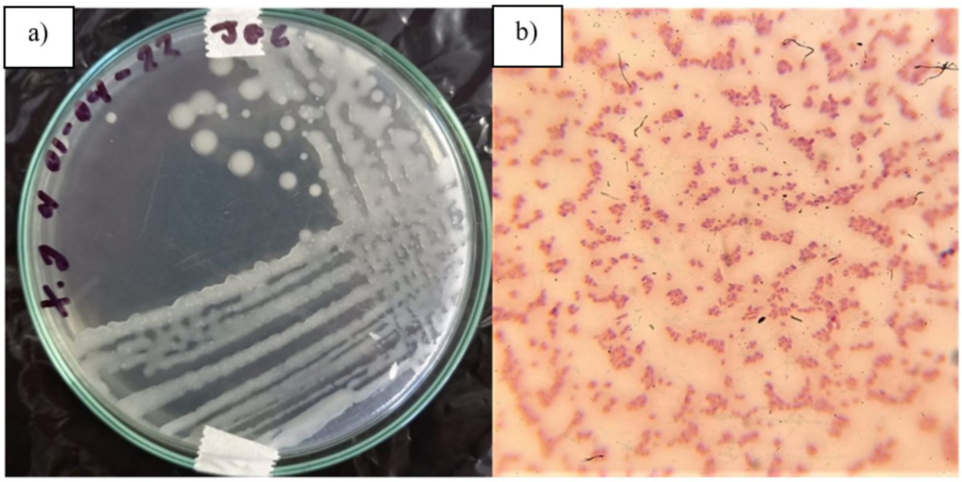

Figure 3a shows yellow colonies shaped like a "fried egg" with various amounts of slime production under specific conditions of the culture medium. And in **Figure 3b**, short, Gram-negative rod, polymorphic and branched in some cases, were observed by Gram staining.

Disinfection of *P. vulgaris, C. sativus,* and *S. lycopersicum* seeds

P. vulgaris, C. sativus, and *S. lycopersicum* seeds were disinfected with 5% (v/v) NaClO for 5 min, washed five times with sterile water, and then with 70% (v/v) alcohol for 5 min, washed five times with sterile water,[1,17], as it is shown in Figure 1.

Obtaining the crude carbon nanoparticle extract from *Albizia* sp

Albizia sp leaves were collected from Ciudad Universitaria, UMSNH, Morelia, Mich., México and disinfected by immersion in 0.5% NaCl for 1 min, rinsed with sterile deionized water, then cut into 5.0 cm pieces with sterile scissors and dried at 80°C for 12 h. 30 g of *Albizia* sp were used, suspended in 300 mL of deionized water, which was heated to 70°C for 30 min. The aqueous extract of *Albizia* sp was filtered through Whatman No. 1 paper and centrifuged at 4000 rpm for 10 min. The supernatant was refrigerated at 4°C,[9,10,12] as it is shown in Figure 1.

Inoculation of *A. vinelandii* and/or *X. autotrophicus* in *P. vulgaris, C. sativus* and *S. lycopersicum* seeds and addition of CCNPE

In 250 g plastic bags, for every 10 *P. vulgaris, C. sativus* and *S. lycopersicum* seeds, 1.0 mL (v/v) of *A. vinelandii* and/or *X. autotrophicus* were inoculated in a 1:1 (v/v) ratio, equivalent to a concentration of *A. vinelandii* with 1.17 X 10^9 CFU/mL and *X. autotrophicus* with 1.59 X 10^6 CFU/mL calculated by viable count on Burk agar and Nutrient agar; then, both of them were treated with 1.0 mL of a concentration of 10 ppm and/or 20 ppm of CCNPE in 0.85% SSD. Seeds with *A. vinelandii* and/or *X. autotrophicus* treated with CCNPE were shaken at 200 rpm/30 min at 28°C and sown in Leonard's jar soil according to the experimental design in Table 1 with 2 controls, 6 treatments and 6 replicates: *P. vulgaris, C. sativus* and *S. lycopersicum* irrigated with water only or absolute control (AC); *P. vulgaris, C. sativus* and *S. lycopersicum* with 100% NH_4NO_3 uninoculated or relative control (RC); *P. vulgaris, C. sativus* and *S. lycopersicum* with *A. vinelandii* and/or *X. autotrophicus* enhanced with 10 and/or 20 ppm of CCNPE and 50% NH_4NO_3. NH_4NO_3 in the mineral solution was applied every third day for one month.[17] The response variables used were: days to emergence and 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) at seedling level,[1,17,18] as it is shown in Figure 1.

Table 1 Experimental design to analyze the response of *P. vulgaris, C. sativus* and *S. lycopersicum* to *A. vinelandii* and/or *X. autotrophicus* at dose 50% of NH_4NO_3 plus crude carbon nanoparticles extract

Treatments** ***P. vulgaris /C.sativus/ S. lycopersicum	***Azotobacter vinelandii***	***Xanthobacter autotrophicus***	**crude carbon nanoparticles extract (ppm)**	**NH_4NO_3**
(AC) Absolute control or water	-	-	-	-
(RC) Relative control	-	-	-	100 %
T1	+	-	10	50 %
T2	-	+	10	50 %
T3	+	+	10	50 %
T4	+	-	20	50 %
T5	-	+	20	50 %
T6	+	+	20	50 %

*number of repetitions (n) = 6; (+) = applied, (-) = not applied.

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Statistical analysis

The experimental data were subjected to ANOVA using Tukey's HSD test ($P < 0.05$) using the statistical software Statgraphics Centurion.

Results & discussion

Table 2 shows *C. sativus* with *A. vinelandii* and *X. autotrophicus* with 20 ppm of CCNPE and 50% NH_4NO_3, that reached 98.28% germination, a numerical value with statistical difference compared to 26.19% germination of *C. sativus* uninoculated either CCNPE, fed with 100% NH_4NO_3 or relative control (RC); and with 45.71% germination of *C. sativus* with *X. autotrophicus* 50% NH_4NO_3 and 20 ppm of CCNPE. These results indirectly support that the *C. sativus* seed, when imbibing water, initiated the hydrolysis of starch by α-amylase, that generated the release of organic acids, amino acids and glucose by the degradation of the endosperm, that were transformed by *A. vinelandii* and *X. autotrophicus* into phytohormones that induced the rapid uptake of 50% NH_4NO_3, enhanced with CCNPE to achieve a higher percentage of germination.[2,5,7] This fact was confirmed in the germination of the *C. sativus* seed with *A. vinelandii* and *X. autotrophicus* with 50% NH_4NO_3 and CCNPE; there, better growth was observed in the root and seedling primordium on the 4th day of emergence as shown in Figure 4.

Table 2 Effect of *Azotobacter vinelandii* and *Xanthobacter autotrophicus* at 50% NH_4NO_3 plus CCNPE on the germination of *Cucumis sativus*

****C. sativus* seeds**	**Days of emergency**	**Germination percentage (%)**
Absolute control irrigated only water	7^{c**}	26.19^{d}
Relative control fed at NH_4NO_3 100% uninoculated	6^{b}	45.71^{c}
A. vinelandii at 50% NH_4NO_3 plus 10 ppm CCNPE	6^{b}	48.52^{c}
A. vinelandii at 50% NH_4NO_3 plus 20 ppm CCNPE	6^{b}	63.33^{b}
X. autotrophicus at 50% NH_4NO_3 plus 10 ppm CCNPE	5^{a}	64.29^{b}
X. autotrophicus at 50% NH_4NO_3 plus 20 ppm CCNPE	5^{a}	68.04^{b}
A. vinelandii + *X. autotrophicus* at 50% NH_4NO_3 plus 10 ppm CCNPE	4^{a}	89.52^{a}
A. vinelandii + *X. autotrophicus* at 50% NH_4NO_3 plus 20 ppm CCNPE	4^{a}	98.28^{a}

*n=6, crude carbon nanoparticle extract (CCNPE); **values with different letters had statistical differences (P<0.05) according to ANOVA/Tukey.

Figure 4 Effect of *Azotobacter vinelandii* and *Xanthobacter autotrophicus* at 50% NH_4NO_3 plus crude carbon nanoparticle extract (CCNPE) on the germination of *Cucumis sativus* 7 days after sowing.

AC= *C. sativus* uninoculated irrigated with water; RC= *C. sativus* uninoculated or either treated with CCNPE fed with 100% NH_4NO_3; T1= *C. sativus* + *A. vinelandii* + 50% NH_4NO_3 + 10 ppm CCNPE; T2= *C. sativus* + *A. vinelandii* + 50% NH_4NO_3 +20 ppm CCNPE; T3= *C. sativus* + *X. autotrophicus* + 50% NH_4NO_3 + 10 ppm CCNPE; T4= *C. sativus* + *autotrophicus* + 50% NH_4NO_3+ 10 ppm CCNPE; T6= *C. sativus* + *A. vinelandii/X. autotrophicus* + 50% NH_4NO_3+ 20 ppm CCNPE.

Table 3 shows *P. vulgaris* with *A. vinelandii* with 50% NH_4NO_3 enhanced with 20 ppm CCNPE, that reached 77.85% germination; and in *P. vulgaris* with *A. vinelandii* and *X. autotrophicus* with 50% NH_4NO_3 plus 20 ppm CCNPE with 90.71% germination; both numerical values had statistical difference compared to the 62.85% germination of *P. vulgaris* uninoculated with *A. vinelandii* and/or *X. autotrophicus* nor treated with CCNPE, fed with 100% NH_4NO_3 or RC. These results are similar to those reported in literature;[2,4,5,9] when treating *T. aestivum* seeds with *B. thuringiensis* and *X. autotrophicus*50% NH_4NO_3 plus 20 ppm of CCNPE, that reported up to 93% and 86% germination of *T. aestivum* seeds. This is confirmed in Figure 5, that shows the positive response of *A. vinelandii* and *X. autotrophicus* enhanced with 50% NH_4NO_3 in germination between 4 and 8 days after sowing.

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Table 3 Effect of *Azotobacter vinelandii* and *Xanthobacter autotrophicus* on days to emergence and germination percentage of *Phaseolus vulgaris* with 50% NH_4NO_3 plus crude carbon nanoparticle extract

*** Phaseolus vulgaris**	**Days of emergency**	**Germination percentage (%)**
(AC) Absolute control	8^{d**}	60.71^{b}
(RC) Relative control 100 % NH_4NO_3 100%	7^{c}	62.85^{b}
A.vinelandii + 50% NH_4NO_3 + 10 ppm CCNPE	6^{b}	81.42^{b}
A.vinelandii + 50% NH_4NO_3 + 20 ppm CCNPE	6^{a}	77.85^{a}
X. autotrophicus + 50% NH_4NO_3 10 ppm CCNPE	6^{b}	71.42^{b}
X. autotrophicus + 50% NH_4NO_3 + 20 ppm CCNPE	5^{a}	80.71^{b}
A.vinelandii + *X. autotrophicus* + 50% NH_4NO_3 + 10 ppm CCNPE	5^{a}	81.42^{b}
A.vinelandii + *X. autotrophicus* + 50% NH_4NO_3 20 ppm CCNPE	4^{a}	9.71^{a}

*n=6, crude carbon nanoparticle extract (CCNPE); **values with different letters had statistical differences (P<0.05) according to ANOVA/Tukey.

Figure 5 Effect of *Azotobacter vinelandii* and *Xanthobacter autotrophicus* with 50% NH_4NO_3 and crude carbon nanoparticle extract (CCNPE) crude carbon nanoparticle extract on the germination percentage of *Phaseolus vulgaris* 8 days after sowing.

AC= *P. vulgaris* uninoculated irrigated with water; RC= *P. vulgaris* uninoculated with 100% NH_4NO_3 or CCNPE: T1= *P. vulgaris* + *A. vinelandii* + 50% NH_4NO_3 + 10 ppm CCNPE; T2= *P. vulgaris* + *A. vinelandii* + 50% NH_4NO_3+ 20 ppm CCNPE + T3= *P. vulgaris* + *X. autotrophicus* + 50% NH_4NO_3 10 ppm CCNPE; T4= *P. vulgaris* + *X. autotrophicus* + 50% NH_4NO_3 plus 20 ppm CCNPE. T5= *P. vulgaris* + *A. vinelandii/X. autotrophicus* + 50% NH_4NO_3 plus 10 ppm CCNPE; T6= *P. vulgaris* + *A. vinelandii/X. autotrophicus* + 50% NH_4NO_3+ 20 ppm CCNPE

In Table 4 shows, *S. lycopersicum* with *X. autotrophicus* 50% NH_4NO_3 enhanced with 20 ppm CCNPE registered 100% germination of *S. lycopersicum* uninoculated with *A. vinelandii* and *X. autotrophicus* either fed with 100% NH_4NO_3 without CCNPE; both numerical values had statistical difference compared to 80% germination of non-inoculated *C. sativus,* irrigated only with water, without CCNPE or absolute control (AC). These results show the positive response of *S. lycopersicum* seeds to imbibe water, this plant activated α-amylase that catalyzed the hydrolysis of starch, released organic acids by degrading the endosperm of the seed, that *A. vinelandii* and *X. autotrophicus* transformed into phytohormones, that shortened the days of emergence and increased the germination of *S. lycopersicum.*[7,9,18,19] The above confirms that the germination of the *S. lycopersicum* seed with *A. vinelandii* and *X. autotrophicus* 50% NH_4NO_3 and CCNPE; there a greater growth was observed in the root primordium, and seedling on the 5th day of emergence as seen in Figure 6.[18,19]

Table 4 Effect of *Azotobacter vinelandii* and *Xanthobacter autotrophicus* on the days to emergence and germination percentage of *Solanum lycopersicum* with NH_4NO_3 50% and CCNPE

***Solanum lycopersicum**	**Days of germination**	**Germination percentage (%)**
(AC) absolute control irrigated water	6^{b**}	80^{c}
(RC) relative control NH_4NO_3 100%	5^{a}	100^{a}
A.vinelandii + 50% NH_4NO_3 10 ppm CCNPE	5^{a}	92.38^{b}
A.vinelandii + NH_4NO_3 50% +20 ppm CCNPE	5^{a}	79.04^{c}
X. autotrophicus + NH_4NO_3 50%+ 10 ppm CCNPE	6^{b}	57.14^{d}
X. autotrophicus + + NH_4NO_3 50% + CCNPE 20 ppm	5^{a}	100^{a}
A.vinelandii + *X. autotrophicus* + NH_4NO_3 50% + 10 ppm CCNPE	6^{b}	68.57^{d}
A.vinelandii + *X. autotrophicus* + NH_4NO_3 50% + 20 ppm CCNPE	6^{b}	82.86^{c}

*n=6, crude carbon nanoparticle extract (CCNPE); **values with different letters had statistical differences (P<0.05) according to ANOVA/Tukey.

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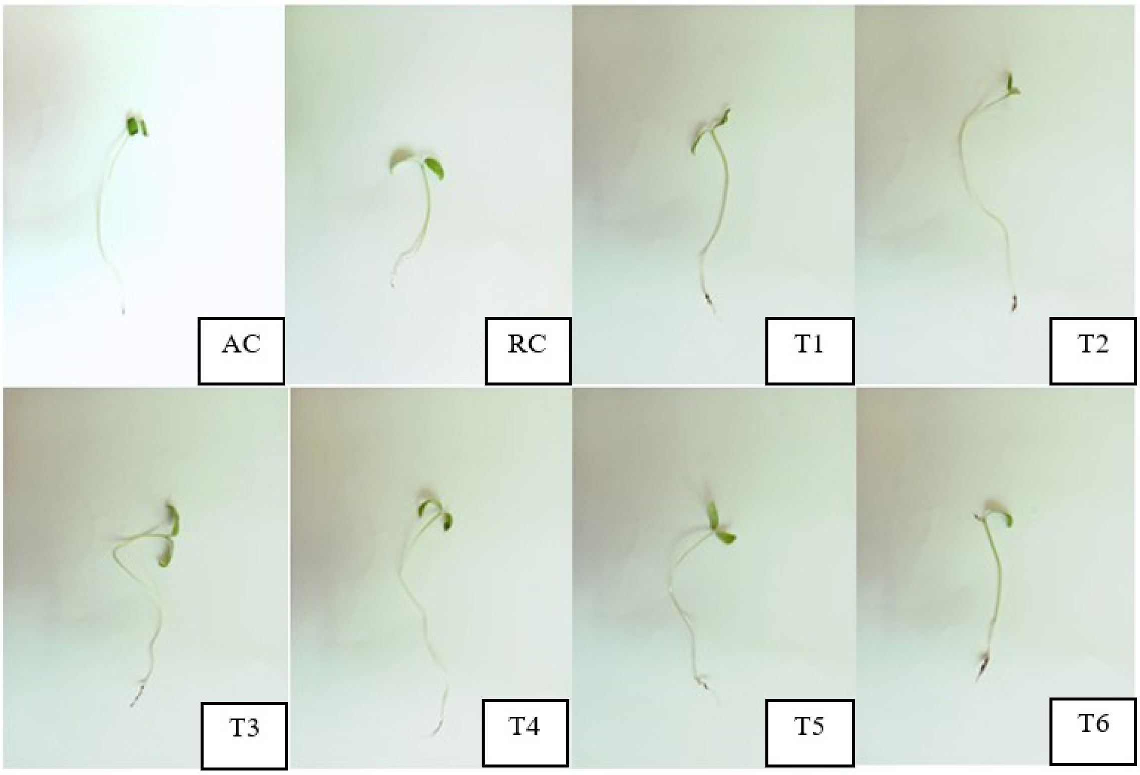

Figure 6 Effect of *Azotobacter vinelandii and Xanthobacter autotrophicus* on the germination of *Solanum lycopersicum* with 50% NH_4NO_3 plus crude carbon nanoparticle extract (CCNPE) at 6 days after snowing.

AC= *S. lycopersicum* uninoculated irrigated with water; RC= *S. lycopersicum* fed with 100% NH_4NO_3 uninoculated nor CCNPE:T1= *S. lycopersicum* + *A. vinelandii* + fed with 50% NH_4NO_3+ 10 ppm CCNPE; T2= *S. lycopersicum* + *A. vinelandii* + 50% NH_4NO_3+ 20 ppm CCNPE; T3= *S. lycopersicum* + *X. autotrophicus* + 50% NH_4NO_3 + 10 ppm CCNPE; T4= *S. lycopersicum* + *X. autotrophicus* + 50% NH_4NO_3 + 20 ppm CCNPE; T5= *S. lycopersicum* + *A. vinelandii/X. autotrophicus* + 50% NH_4NO_3+ 10 ppm CCNPE ; T6= *S. lycopersicum* + *A. vinelandii/X. autotrophicus* + 50% NH_4NO_3 + 20 ppm CCNPE.

In Table 5, the positive effect of *A. vinelandii* and *X. autotrophicus* on *C. sativus* with 50% NH_4NO_3 plus 10 ppm CCNPE per seedling is shown, that registered 14.14 cm of PH and 6.68 cm of RL, these numerical values had not statistical difference compared to 14.33 cm of PH and 2.16 of *C. sativus* with 100% NH_4NO_3 or (RC) uninoculated, nor CCNPE with numerical values registered in *C. sativus* with *A. vinelandii* and *X. autotrophicus* 50% NH_4NO_3 plus 10 and 20 ppm of CCNPE. In fresh and dry biomass *P. vulgaris* with *A. vinelandii* and *X. autotrophicus* at 50% NH_4NO_3+ 10 ppm CCNPE registered 0.33g AFW, 0.0102g RFW, 0.0116g ADW and 0.0149g RDW. These values were either had not statistically different from the 0.47g AFW, 0.012g RFW, 0.0241g ADW and 0.0018g RDW of *P. vulgaris* 100% NH_4NO_3 non-inoculated with either CCNPE or RC. Although no differences were registered it was observed that, *A. vinelandii* and *X. autotrophicus* in *C. sativus* with 50% NH_4NO_3 plus 10 ppm of CCNPE, in that sense *A. vinelandii* and *X. autotrophicus*, to convert the organic compounds of photosynthesis into phytohormones, this support that CCNPE, improved the activity for greater proliferation of secondary roots and maximize the radical uptake of 50% NH_4NO_3, without negative effect on the growth of *C. sativus*.[20–23] This is confirmed by what was observed in Figure 6, where the trend of a positive effect of *A. vinelandii* and *X. autotrophicus* on *C. sativus* is observed, with 50% NH_4NO_3 plus CCNPE at 14 days after sowing, where a tendency towards an increase in stem diameter and root density was registered compared,[24–26] to *C. sativus* used as RC, where stem diameter and root density were apparently lower, despite not being statistically difference an inclination is observed that 100% NH_4NO_3 was not uptake to the maximum, shown on Figure 7.

Table 5 Effect of *Azotobacter vinelandii* and *Xanthobacter autotrophicus* on the phenology and biomass of *Solanum lycopersicum* with 50% NH_4NO_3 plus crude carbon nanoparticles extract

	Phenology		**Biomass**			
*Treatment *S. lycopersicum*	Plant height (cm)	Radical length (cm)	Aerial fresh weight (g)	Radical fresh weight (g)	Aerial dry weight (g)	Radical Dry weight (g)
(AC) Absolute control Irrigated water	5.5[c**]	1.42[d]	0.092[d]	0.004[c]	0.0125[d]	0.0042[b]
(RC) Relative control fed % 100 NH_4NO_3	14.33[a]	2.16[c]	0.47[a]	0.012[b]	0.0241[b]	0.0018[c]
A. *vinelandii* , 50% NH_4NO_3 + 10 ppm CCPNE	12.11[b]	6.22[a]	0.38[b]	0.033[a]	0.0279[b]	0.0038[b]

Citation: Castillo JO, Cruz JLI, Perez-González DA, et al. Growth of *Cucumis sativus, Phaseolus vulgaris and Solanum lycopersicum with Azotobacter vinelandii and Xanthobacter autotrophicus* at 50% NH4NO3 plus crude carbon nanoparticle extract reduces N2O release in soil. *Open Access J Sci.* 2025;8(1):257‒267. DOI: 10.15406/oajs.2025.08.00270

A.vinelandii, 50 % NH_4NO_3 + 20 ppm CCPNE	10.87^b	5.37^b	0.41^a	0.028^a	0.0265^b	0.0034^b
X. autotrophicus 50 % NH_4NO_3 + 10 ppm CCPNE	10.75^b	7.3^a	0.30^b	0.004^c	0.0142^c	0.0109^a
X. autotrophicus, 50 % NH_4NO_3 + 20 ppm CCPNE	12.43^b	4.58^b	0.23^c	0.028^a	0.0514^a	0.0054^b
A. vinelandii + *X. autotrophicus*, 50 % NH_4NO_3+ 10 ppm CCPNE	14.14^a	6.68^a	0.33^b	0.0102^b	0.011^6d	0.014^9a
A. vinelandii + *X. autotrophicus* 50 % NH_4NO_3 +20 ppm CCPNE	12.43^b	6.76^a	0.32^b	0.0125^a	0.0307^a	0.0099^a

*n=6, crude carbon nanoparticle extract (CCNPE)**Values with different letters had statistical difference (P<0.05) according to ANOVA-Tukey

Figure 7 Effect of *Azotobacter vinelandii* and *Xanthobacter autotrophicus* with 50% NH_4NO_3 and crude carbon nanoparticle extract (CCNPE) on the phenology and biomass of *Cucumis sativus* at seedling stage.

AC= *C. sativus* uninoculated irrigated with water; RC= *C. sativus* with 100% NH_4NO_3 inoculated , nor CCNPE:T1= *C. sativus* + *A. vinelandii* + 50% NH_4NO_3 + 10 ppm CCNPE;T2= *C. sativus* + *A. vinelandii* + 50% NH_4NO_3 + 20 ppm CCNPE;T3= *C. sativus* + *X. autotrophicus* 50% NH_4NO_3 + 10 ppm CCNPE;T4= *C. sativus* + *X. autotrophicus* 50% NH_4NO_3 + 20 ppm CCNPE ;T5= *C. sativus* + *A. vinelandii/X. autotrophicus* + 50% NH_4NO_3 + 10 ppm CCNPE ;T6= *C. sativus* + *A. vinelandii/X. autotrophicus* + 50% NH_4NO_3 + 20 ppm CCNPE.

Table 6 shows the positive effect of *A. vinelandii* and/or *X. autotrophicus with P. vulgaris* 50% NH_4NO_3 and 10 ppm CCNPE in the pre-flowering stage, that registered 29.33 cm of PH and 21.33 cm of RL, numerical values with statistical difference with respect to the 15.0 cm of PH and 11.0 of *P. vulgaris* with 100% NH_4NO_3 or RC uninoculated either any CCNPE. While numerical values registered in *P. vulgaris* with *A. vinelandii* and *X. autotrophicus* at 50% NH_4NO_3 plus 10 ppm CCNPE, in fresh and dry weight registered 6.88 g AFW, 3.80 g RFW 0.66 g ADW and 0.25 g RDW these values were statistically different from 3.47 g AFW, 1.07 g RFW, 0.32 g ADW and 0.04 g RDW of *P. vulgaris* at 100% NH_4NO_3 or RC not inoculated with *A. vinelandii* nor *X. autotrophicus,* untreated with CCNPE. The positive effect of *A. vinelandii* and *X. autotrophicus* on *P. vulgaris* with 50% NH_4NO_3 indicates that both genera and species of endophytes, when colonizing the interior of the roots of this legume, converted compounds of the root metabolism into phytohormones that optimized the nitrogen fertilizer reduced to 50%, without affecting the healthy growth of *P. vulgaris*, while the CCNPE accelerated the uptake of bacterial phytohormonal activity to avoid NH_4NO_3 remnants that cause air, water or lure contamination.[27–29] This is confirmed by what is observed in Figure 8, where the positive effect of *A. vinelandii* and *X. autotrophicus on P. vulgaris* with 50% NH_4NO_3 and the CCNPE at 25 days after planting, showed an increase in stem diameter and root density compared, to *P. vulgaris* or RC where there was a lower stem diameter and root density, making it evident that 100% NH_4NO_3 was not uptake to the maximum due, to a natural deficiency of *P. vulgaris,* that can only be corrected with endophytic invasion with *A. vinelandii* and *X. autotrophicus,* to increase the capacity of the root system for a maximum uptake of 50% NH_4NO_3 (30-31), while the CCNPE accelerated both the generation of bacterial phytohormones and the speed of uptake of nitrogen fertilizer to avoid environmental pollution and reduce N_2O generation.[14,20, 25,28]

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Table 6 Effect of *Azotobacter vinelandii* and *Xanthobacter autotrophicus* on the phenology and biomass of *Phaseolus vulgaris* with 50% NH_4NO_3 and a crude carbon nanoparticle extract or CCNPE at pre-flowering stage

	Phenology		Biomass			
*Treatment *P. vulgaris*	Plant height (cm)	Radical length (cm)	Aerial fresh weight (g)	Radical fresh weight (g)	Aerial dry weight (g)	Radical dry weight (g)
(AC) absolute control irrigated water	11.8[b**]	6.6[c]	1.60[e]	0.48[c]	0.15[c]	0.03[d]
(RC) relative control fed % 100 NH_4NO_3	15.0[b]	11.0[c]	3.47[c]	1.07[b]	0.32[b]	0.04[c]
A. vinelandii + 50 % NH_4NO_3 + 10 ppm CCNPE	23.75[b]	15.75[b]	4.71[b]	1.14[b]	0.44[b]	0.06[c]
A.vinelandii + 50 % NH_4NO_3 + 20 ppm CCPNE	26.33[a]	14.0[b]	4.41[b]	1.58[b]	0.42[b]	0.10[b]
X. autotrophicus 50 % NH_4NO+ 10 ppm CCNPE	20.0[b]	15.0[b]	3.39[c]	1.65[b]	0.33[b]	0.22[a]
X. autotrophicus + 50 % NH_4NO_3 + 20 ppm CCNPE	23.023[b]	14.5[b]	4.22[b]	1.00[b]	0.37[b]	0.05[c]
A. vinelandii + *X. autotrophicus* +50 % NH_4NO_3 +10 ppm CCNPE	29.33[a]	21.33[a]	6.88[a]	3.80[a]	0.66[a]	0.25[a]
A. vinelandii + *X. autotrophicus* +50 % NH_4NO_3 + 20 ppm CCNPE	31.33[a]	12[bc]	5.55[a]	2.07[a]	0.54[a]	0.13[a]

*n=6, crude carbon nanoparticle extract (CCNPE)**Values with different letters had statistical difference (P<0.05) according to ANOVA-Tukey

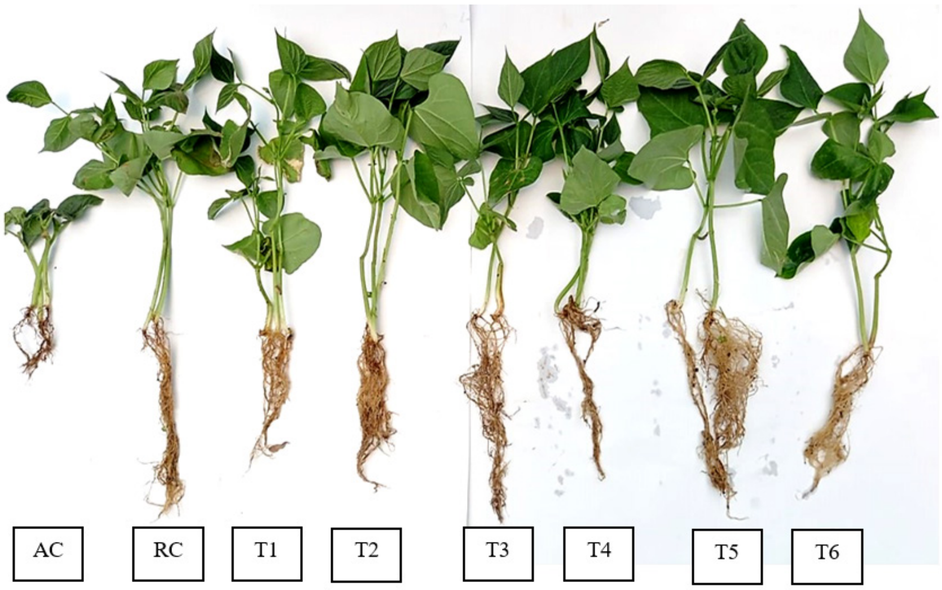

Figure 8 Effect of *Azotobacter vinelandii* and *Xanthobacter autotrophicus* with 50% NH_4NO_3 plus crude carbon nanoparticle extract (CCNPE) on the phenology and biomass of *Phaseolus vulgaris* at the seedling level 23 days after sowing.

AC= *P. vulgaris* uninoculated irrigated with water; RC= *P. vulgaris* uninoculated with 100% NH_4NO_3 or CCNPE:T1= *P. vulgaris* + *A. vinelandii* + 50% NH_4NO_3 + 10 ppm CCNPE;T2= *P. vulgaris* + *A. vinelandii* + 50% NH_4NO_3+ 20 ppm CCNPE + T3= *P. vulgaris* + *X. autotrophicus* + 50% NH_4NO_3 10 ppm CCNPE;T4= *P. vulgaris* + *X. autotrophicus* + 50% NH_4NO_3 plus 20 ppm CCNPE. T5= *P. vulgaris* + *A. vinelandii/X. autotrophicus* + 50% NH_4NO_3 plus 10 ppm CCNPE;T6= *P. vulgaris* + *A. vinelandii/X. autotrophicus* + 50% NH_4NO_3+ 20 ppm CCNPE

Table 7 and Figure 9 and 10 show the yield elements of *P. vulgaris* with *A. vinelandii* plus *X. autotrophicus* with 50% NH_4NO_3 and the CCNPE. It was evident that the maximum average value in centimeters of the pods were those of *P. vulgaris* inoculated individually with *A. vinelandii* 10.4 cm or *X. autotrophicus* 10.3 cm, or the mixture of both with 10.75 cm, with 50% NH_4NO_3 and 10 ppm of CCNPE, which shows that the ability of these genera and endophytic species to invade the root system ensures that both *A. vinelandii and X. autotrophicus* transform compounds of the root metabolism of *P. vulgaris* into phytohormones that increase the uptake of NH_4NO_3 to 50%, while the CCNPE accelerates and optimizes the uptake of this nitrogen fertilizer to the máximum,[31–35] thereby avoiding a remainder of NH_4NO_3 that causes the mineralization of the soil organic matter reserve, at the same time there is NH_4NO_3 that is converted into N_2O, decreasing the generation of this greenhouse gas, global warming is avoided.[2,4] While the average values of the pod size of *P. vulgaris* as well as the fresh and dry weight, indicate that there was no negative effect on the productive capacity of *P. vulgaris*, while the numerical values were different from those registered in *P. vulgaris* not inoculated with 100% NH_4NO_3, without CCNPE with 8.0 cm and when *P. vulgaris* with *A. vinelandii* and *X. autotrophicus* with 50% NH_4NO_3, with 20 ppm of CCNPE with 8.21 cm. The above supports that the application of *A. vinelandii* and *X. autotrophicus* with the reduction of the dose of NH_4NO_3, and CCNPE are useful for agricultural production without risk of environmental pollution or N_2O.[5,9,12,29]

Table 7 Yield elements of *Phaseolus vulgaris* with *Azotobacter vinelandii* and *Xanthobacter autotrophicus* 50% NH_4NO_3 with a crude carbon nanoparticle extract or CCNPE

Treatment/ *P. vulgaris	**Average fruit size (cm)**	**Averge fruit fresh weight (g)**	**Average fruit dry weight (g)**
(RC) Relative control at 100 % NH_4NO_4	8.0^{b**}	1.22^{b}	0.01^{e}
A. vinelandii + 50 % NH_4NO_3 +10 ppm CCNPE	10.4^{a}	2.08^{a}	0.19^{b}
A. vinelandii + 50 % NH_4NO_3 + 20 ppm CCNPE	7.0^{b}	1.07^{b}	0.16^{c}
X. autotrophicus + 50 % NH_4NO_3 + 10 ppm CCNPE	10.3^{a}	2.06^{a}	0.19^{b}
A. vinelandii + *X. autotrophicus,* 50 % NH_4NO_3 +10 ppm CCNPE	10.75^{a}	2.63^{a}	0.25^{a}
A. *vinelandii* + *X. autotrophicus* + 50 % NH_4NO_3 + 20 ppm CCNPE	8.21^{b}	1.52^{b}	0.04^{d}

*n=12, crude carbon nanoparticle extract (CCNPE) **Values with different letters had statistical difference (P<0.05) according to ANOVA-Tukey

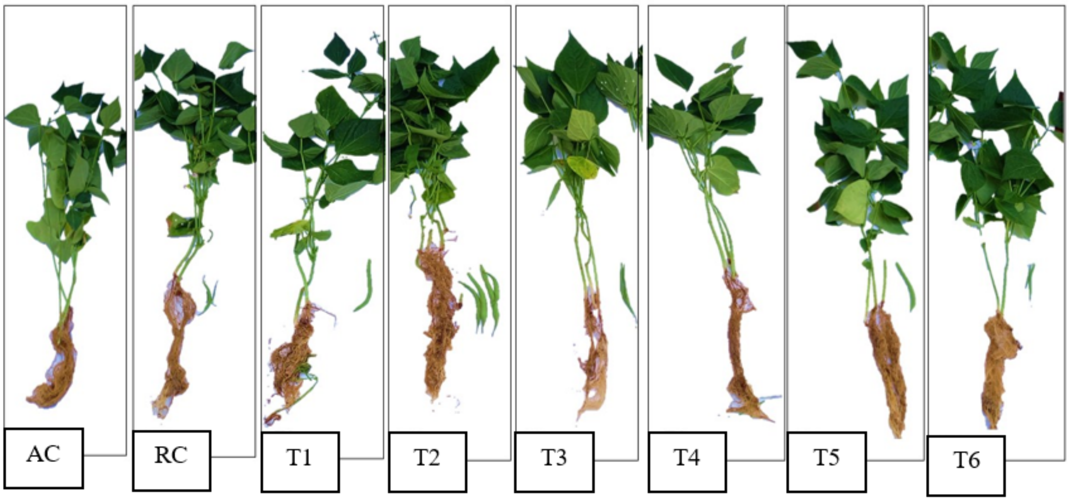

Figure 9 Effect of *Azotobacter vinelandii and Xanthobacter autotrophicus* with 50% NH_4NO_3 and crude carbon nanoparticle extract or CCNPE on the phenology and biomass of *Phaseolus vulgaris* at physiological maturity level 57 days after sowing.

AC= *P. vulgari*s uninoculated irrigated with water; RC= *P. vulgaris* uninoculated with 100% NH_4NO_3 or CCNPE:T1= *P. vulgaris* + *A. vinelandii* + 50% NH_4NO_3 + 10 ppm CCNPE;T2= *P. vulgaris* + *A. vinelandii* + 50% NH_4NO_3+ 20 ppm CCNPE + T3= *P. vulgaris* + *X. autotrophicus* + 50% NH_4NO_3 10 ppm CCNPE;T4= *P. vulgaris* + *X. autotrophicus* + 50% NH_4NO_3 plus 20 ppm CCNPE. T5= *P. vulgaris* + *A. vinelandii/X. autotrophicus* + 50% NH_4NO_3 plus 10 ppm CCNPE;T6= *P. vulgaris* + *A. vinelandii/X. autotrophicus* + 50% NH_4NO_3+ 20 ppm CCNPE

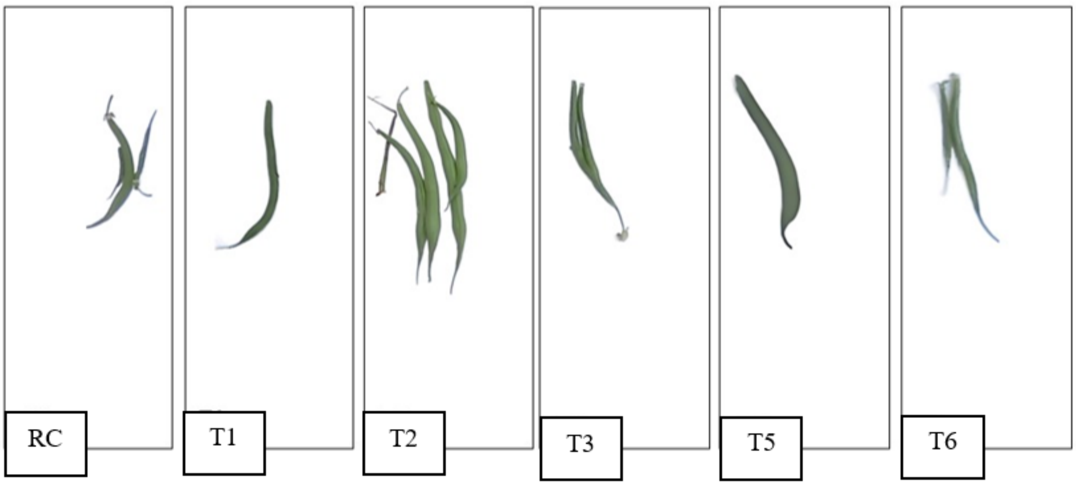

Figure 10 Yield elements of *Phaseolus vulgaris* with *Azotobacter vinelandii and Xanthobacter autotrophicus* 50% NH_4NO_3 plus a crude carbon nanoparticle extract or CCNPE.

AC= *P. vulgari*s uninoculated irrigated with water; RC= *P. vulgaris* uninoculated with 100% NH_4NO_3 either CCNPE:T1= *P. vulgaris* + *A. vinelandii* + 50% NH_4NO_3 + 10 ppm CCNPE;T2= *P. vulgaris* + *A. vinelandii* + 50% NH_4NO_3+ 20 ppm CCNPE + T3= *P. vulgaris* + *X. autotrophicus* + 50% NH_4NO_3 10 ppm CCNPE;T4= *P. vulgaris*

Citation: Castillo JO, Cruz JLI, Perez-González DA, et al. Growth of *Cucumis sativus, Phaseolus vulgaris and Solanum lycopersicum with Azotobacter vinelandii and Xanthobacter autotrophicus* at 50% NH4NO3 plus crude carbon nanoparticle extract reduces N2O release in soil. *Open Access J Sci.* 2025;8(1):257‒267. DOI: 10.15406/oajs.2025.08.00270

+ *X. autotrophicus* + 50% NH_4NO_3 plus 20 ppm CCNPE. T5= *P. vulgaris* + *A. vinelandii/X. autotrophicus* + 50% NH_4NO_3 plus 10 ppm CCNPE; T6= *P. vulgaris* + *A. vinelandii/X. autotrophicus* + 50% NH_4NO_3+ 20 ppm CCNPE

Conclusion

It was evident that the inoculation of seeds of *C. sativus, P. vulgaris* and *S. lycopersicum* with the endophytes *A. vinelandii* and/or *X. autotrophicus* with 50% NH_4NO_3 and a CCNPE favored in all plants the maximum uptake of NH_4NO_3, accelerated by the addition of CCNPE without affecting the healthy growth of each plant, while the maximum uptake of NH_4NO_3, avoided the generation of N_2O, as well as the loss of organic matter, contamination with NO_3 remaining in water and soil. The above is an example of a strategy in agriculture to mitigate global warming.

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Conflicts of interest

The authors declare no conflicts of interest.

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