

# Optimization of $\text{NH}_4\text{NO}_3$ to 50% and healthy growth of *Phaseolus vulgaris* and *Triticum aestivum* with *Methylobacterium symbioticum* and *Xanthobacter autotrophicus* and a crude carbon nanoparticle extract

## Abstract

The intensive cropping of *Phaseolus vulgaris* and *Triticum aestivum* requires nitrogen fertilizer such as  $\text{NH}_4\text{NO}_3$  for healthy growth. However,  $\text{NH}_4\text{NO}_3$  normally exceeds the uptake capacity of the root system of both plant species, leaving  $\text{NO}_3^-$  behind. The remainder in the soil becomes the final electron acceptor for the anaerobic heterotrophic microbiota, releasing nitrogen oxide ( $\text{N}_2\text{O}$ ), a greenhouse gas that contributes to global warming. Therefore, the objective of this research was to analyze the effect of *M. symbioticum* and *X. autotrophicus* on the growth of *P. vulgaris* and *T. aestivum* with 50%  $\text{NH}_4\text{NO}_3$  and 10 ppm a crude carbon nanoparticles extract. For this purpose, seeds of *P. vulgaris* and *T. aestivum* were inoculated with *M. symbioticum* and *X. autotrophicus* with 10 ppm of a crude carbon nanoparticle extract (CCNE), in soil contained in Leonard jars poor in organic matter and mineral nitrogen, under greenhouse conditions. The experimental design was: 1) *P. vulgaris* and *T. aestivum* seeds uninoculated irrigated only with water, 2) *P. vulgaris* and *T. aestivum* seeds uninoculated fed with mineral solution and  $\text{NH}_4\text{NO}_3$  at 100%, 3) *P. vulgaris* and *T. aestivum* with *M. symbioticum*, a mineral solution containing 50%  $\text{NH}_4\text{NO}_3$  plus 10 ppm CCNE 4) *P. vulgaris* and *T. aestivum* with *X. autotrophicus* mineral solution and  $\text{NH}_4\text{NO}_3$  at 50% plus 10 ppm CCNE 5) *P. vulgaris* and *T. aestivum* with *M. symbioticum* and *X. autotrophicus* mineral solution with  $\text{NH}_4\text{NO}_3$  at 50% plus 10 ppm CCNE. By the following response variables: phenology: plant height, and root length, biomass: fresh and dry weight of the foliar and root system. Experimental results were analyzed by variance (ANOVA) and Tukey's HSD test ( $p < 0.05$ ).

The results showed a positive effect of *M. symbioticum* and *X. autotrophicus* on the phenology and biomass of *P. vulgaris* and *T. aestivum*, with numerical values statistically difference, even better improvement due to double inoculation with *M. symbioticum* and *X. autotrophicus* was performed on both plant species compared to the growth of *P. vulgaris* and *T. aestivum* uninoculated with mineral solution with 100%  $\text{NH}_4\text{NO}_3$ . This demonstrates that both endophytic bacterial species, resident in the roots of *P. vulgaris* and *T. aestivum*, optimized uptake  $\text{NH}_4\text{NO}_3$  at 50% due to synthesis of phytohormones from root metabolites, while the 10 ppm of CCNE activated rapid  $\text{NH}_4\text{NO}_3$  uptake and a positive response from the root system of *P. vulgaris* and *T. aestivum*, that reduced at minimal concentration of  $\text{NO}_3^-$ , that cause the release of  $\text{N}_2\text{O}$ , that contributes to global warming. This supports the value of *M. symbioticum* and *X. autotrophicus* with the CCNE. *P. vulgaris* and *T. aestivum* without the common environmental detriment of  $\text{NH}_4\text{NO}_3$  over-fertilization.

**Keywords:** soil, legume, cereal, plant growth promoting endophytes, nitrogen fertilizer, global warming, environmental health

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## Introduction

In the cropping of *Phaseolus vulgaris* and *Triticum aestivum* different types of nitrogen fertilizers are used. One of the most common is  $\text{NH}_4\text{NO}_3$ , which, when applied in doses higher than what the plant's root system can uptake, causes a rapid loss of organic matter.<sup>1-4</sup> Some of the  $\text{NH}_4\text{NO}_3$  that is not uptaken causes contamination of surface water and aquifers.<sup>5,6</sup> The physicochemical and biological conditions of the soil induce the formation of  $\text{N}_2\text{O}$ , which contributes as a greenhouse gas to global warming, to the detriment of life on the planet.<sup>7</sup> The current alternative is to reduce the  $\text{NH}_4\text{NO}_3$  dose and inoculate the *P. vulgaris* seed with a genus and species of endophytic

plant growth promoters, as well as be *Rhizobium etli*<sup>8</sup> include less-known species such as *Methylobacterium symbioticum*, that colonizes leaves and roots<sup>9-11,13</sup> or *Xanthomonas autotrophicus*, both of them colonize the interior of *P. vulgaris*.<sup>14-16</sup> These endophytic plant growth promoters can optimize the functioning of the root system, for healthy growth with less  $\text{NH}_4\text{NO}_3$  than recommended,<sup>17,18</sup> especially if a crude carbon nanoparticle extract (CCNE) is added.<sup>7-11</sup> This CCNE enhance mineral uptake and formation of phytohormones, to promote healthy growth of legumes and cereals.<sup>13,17</sup> Therefore, the objective of this work was to analyze the effect of *M. symbioticum* and *X. autotrophicus* on the growth of *P. vulgaris* and *T. aestivum* at 50%  $\text{NH}_4\text{NO}_3$  with 10 ppm of a CCNE.

## Material and methods

This research was conducted at the Environmental Microbiology Laboratory and greenhouse, Institute of Chemical-Biological Research, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, México. The greenhouse was working under the following environmental conditions: a temperature of 23°C, a light intensity of 450  $\mu\text{mol m}^2/\text{s}$ , and a relative humidity of 67%.<sup>18–20</sup>

### Origin of the endophytic bacterial strains applied

*M. symbioticum* was recovery from healthy leaves of *P. vulgaris*. For this, the leaves of *P. vulgaris* were disinfected with 3%  $\text{NaClO}$ /5 min, rinsed 6 times with sterile water, and then ground in a sterile mortar with 0.85% saline solution ( $\text{NaCl}$ ) and 0.1% detergent. Using a sterile 1.0 ml pipette, the resulting aliquots were inoculated into nitrogen-free methanol broth with the following chemical composition (g/L): methanol (after sterilizing the culture medium) 0.1, yeast extract 0.1,  $\text{MgSO}_4$  0.5,  $\text{KCl}$  0.5,  $\text{K}_2\text{HPO}_4$ , 1.0 pH 7.0; it was incubated for 3 days at 30°C and re-cultured on nitrogen-free methanol agar to isolate it. To evaluate the beneficial capacity, seeds of *P. vulgaris* were inoculated with 50%  $\text{NH}_4\text{NO}_3$  to demonstrate the positive effect on  $\text{NH}_4\text{NO}_3$  uptake based on the response variables: percentage of germination, phenology, plant height and root length; biomass, fresh and dry weight of the aerial and root parts, compared with *P. vulgaris* not inoculated with 100%  $\text{NH}_4\text{NO}_3$ . After recovering it from the leaves of inoculated *P. vulgaris*, biochemical and partial molecular identification was carried out to define it as *M. symbioticum* while *X. autotrophicus* donated by Dr. Daniel G Nocera from Department of Chemistry and Chemical Biology of Harvard University, Boston, Ma, USA. These strains were activated on nitrate- and sucrose-free agar for *X. autotrophicus* and with added methanol for *M. symbioticum*, with the following chemical composition (g/L):  $\text{K}_2\text{HPO}_4$  1.0,  $\text{MgSO}_4$  0.5,  $\text{KCl}$  0.5,  $\text{FeSO}_4$  0.5,  $\text{Na}_2\text{MoO}_4$  0.5, pH 6.8, and agar 18.0, adjusted to pH 6.8 then was sterilized at 121°C for 15 minutes, when this culture medium was cooled 1% (v/v) methanol was added at temperature of 45°C. *M. symbioticum* and *X. autotrophicus* were inoculated onto nutrient agar to check purity with the following chemical composition (g/L): 5.0 casein peptone, 3.0 meat extract, pH 7.0, and 18.0 agar.<sup>22–24</sup>

### Experimental used soil

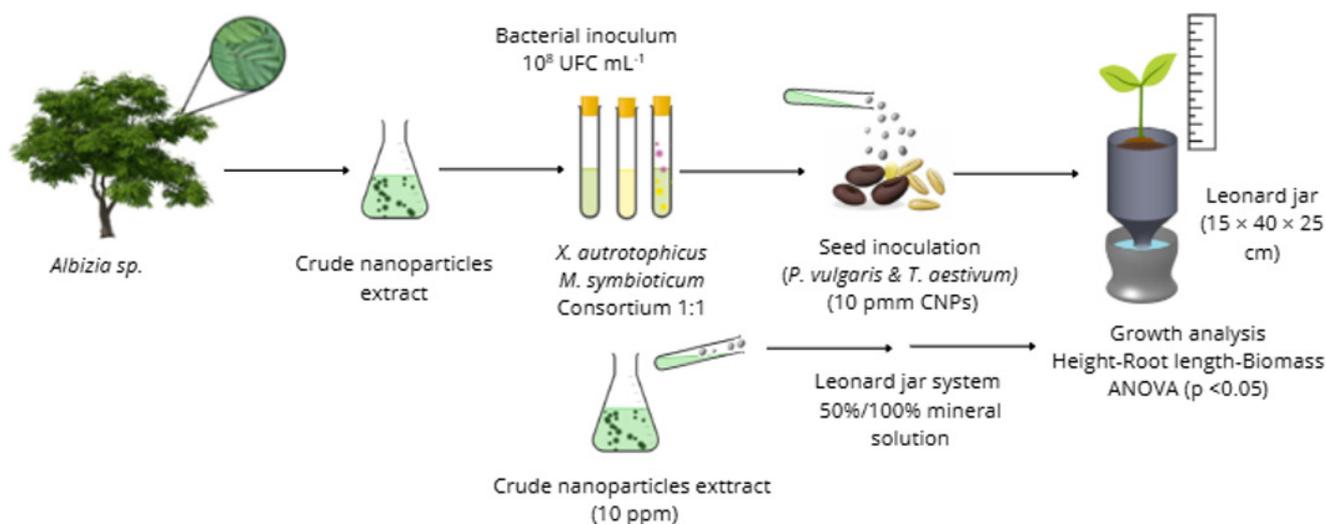
The agricultural soil for this experiment was collected from Tenencia Morelos, Zapata in the municipality of Morelia, Michoacán, México, where *Zea mays* is cropping, located at kilometer 5 of the Morelia–Pátzcuaro highway; it was sieved and solarized for 48 h to reduce the risk of pests and plant diseases, physical chemical analysis indicate that this a sodic lateritic soil with a silty loam texture, poor total nitrogen concentration of 0.10%, poor phosphorus availability of 0.07%, a slightly acidic pH of 6.64, poor organic matter content of 1.20%, poor cation exchange capacity of 10.5, and a field capacity of 30.08%.<sup>25</sup>

## Crude carbon nanoparticles extract

The crude carbon nanoparticle extract was prepared using *Albizia* sp leaves collected from garden of university city of UMSNH, Morelia, Michoacán, México this leaves were disinfected by with 5% sodium hypochlorite solution for 5 min, rinsed 5 times with sterile tap water, then suspended in 0.5%  $\text{NaCl}$  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, then 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, this supernatant was refrigerated at 4°C when no was applied on the experiment.<sup>7–10</sup>

Inoculation of seeds of *P. vulgaris* and *T. aestivum* with *M. symbioticum* and *X. autotrophicus* plus crude carbon nanoparticles extract (CCNE) seeded in Leonard's jar.

To inoculate the seeds of *P. vulgaris* and *T. aestivum*, both strains were suspended in an 18 x 150 mm tube with sterile saline solution at an approximate concentration of  $1.0 \times 10^8$  CFU/mL on the McFarland scale. To inoculate the seeds of *P. vulgaris* and *T. aestivum*, both were disinfected with 5% sodium hypochlorite solution for 5 min, rinsed 5 times with sterile tap water, then disinfected with 70% alcohol for 5 min, and rinsed 6 times with sterile tap water: the seeds were inoculated with bacterial suspensions according to the experimental design (Table 1), at a concentration of  $1 \times 10^8$  CFU/mL to inoculate 100 seeds individually and mixing both of them in 1:1 ratio, then applied at concentration of 10 ppm of CCNE, that were placed in plastic bags where they remained in contact with *M. symbioticum* and *X. autotrophicus* for 30 minutes. Subsequently, 5 seeds were seeded in a semi-hydroponic system or Leonard's jar previously kg of this 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 shown in Figure 1. The mineral solution to fed relative control *P. vulgaris* and *T. aestivum* had following chemical composition g/L:  $\text{NH}_4\text{NO}_3$  10.0,  $\text{K}_2\text{HPO}_4$  2.5,  $\text{KH}_2\text{PO}_4$  2.0,  $\text{MgSO}_4$  1.0,  $\text{NaCl}$  0.1,  $\text{CaCl}_2$  0.1,  $\text{FeSO}_4$  0.01, and 10.0 mL of microelement solution with the chemical composition (g/L):  $\text{H}_3\text{BO}_3$  2.86,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  0.22,  $\text{MgCl}_2 \cdot 7\text{H}_2\text{O}$ . In case of treatments 1 to 3  $\text{NH}_4\text{NO}_3$  concentration was 5.0 g/L. Leonard Jar's (Figure 1) were left in a greenhouse until *P. vulgaris* and *T. aestivum* reached the seedling stage (26–28). The response variables used were: after sowing; phenology: plant height and root length; and biomass: fresh and dry weight of the aerial and root parts of *P. vulgaris* and *T. aestivum*, 21 days after sowing show in experimental design show in Table 1. All experimental results were subjected to analysis of variance (ANOVA) and Tukey's HSD test ( $p < 0.05$ ) using the Statgraphics Centurion XVI statistical package. Figure 1 also shown the isolation, inoculation of *M. symbioticum* and *X. autotrophicus* in *P. vulgaris* and *T. aestivum* with 50%  $\text{NH}_4\text{NO}_3$  and crude carbon extract nanoparticles.



**Figure 1** Isolation, inoculation of *M. symbioticum* and *X. autotrophicus* in *P. vulgaris* and *T. aestivum* with 50%  $\text{NH}_4\text{NO}_3$  and crude carbon extract nanoparticles. (Diagram made by Environmental Microbiology Laboratory-UMSNH).

**Table 1** Experimental design for the analysis of *M. symbioticum* and/or *X. autotrophicus* in *P. vulgaris* and *T. aestivum* with 50%  $\text{NH}_4\text{NO}_3$  plus 10 ppm crude carbon nanoparticles extract

<i>P. vulgaris</i> / <i>T. aestivum</i>	<i>M. symbioticum</i>	<i>X. autotrophicus</i>	Mineral solution with $\text{NH}_4\text{NO}_3$	water	Crude carbon extract nanoparticles 10 ppm
Absolute control (AC) Uninoculated Irrigated water	-	-	100%	+	-
Relative control uninoculated Fed mineral solution $\text{NH}_4\text{NO}_3$ at 100%	-	-	-	-	-
Treatment 1 Fed mineral solution $\text{NH}_4\text{NO}_3$ at 50%	+	-	50%	-	+
Treatment 2 Fed mineral solution $\text{NH}_4\text{NO}_3$ at 50%	-	+	50%	-	+
Treatment 3 Fed mineral solution $\text{NH}_4\text{NO}_3$ at 50%	+	+	50%	-	+

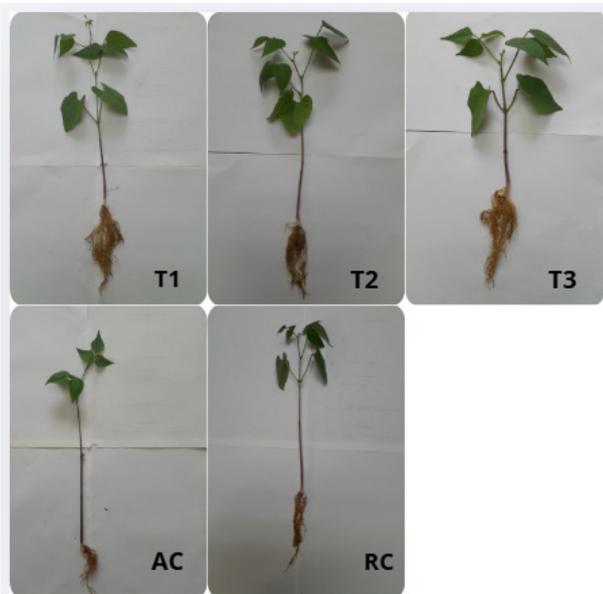
## Results and discussion

Table 2 Effect of *M. symbioticum* and *X. autotrophicus* on growth of *P. vulgaris* with  $\text{NH}_4\text{NO}_3$  at 50% plus 10 ppm crude carbon nanoparticle extract (CCNE) at seedling stage.

<i>Phaseolus vulgaris</i> *	Plant height (cm)	Radical length (cm)	Fresh weight aerial (g)	Fresh weight radical (g)	Dry weight aerial (g)	Dry weight radical (g)
AC=absolute control Uninoculated Irrigated water	9.3 <sup>b</sup>	8.06 <sup>b</sup>	0.865 <sup>d</sup>	0.283 <sup>c</sup>	0.065 <sup>d</sup>	0.027 <sup>d</sup>
RC = relative control Uninoculated Fed mineral solution $\text{NH}_4\text{NO}_3$ at 100%,	10.12 <sup>b</sup>	8.74 <sup>b</sup>	1.116 <sup>c</sup>	0.566 <sup>b</sup>	0.143 <sup>c</sup>	0.058 <sup>c</sup>
<i>X. autotrophicus</i> , mineral solution $\text{NH}_4\text{NO}_3$ at 50% + 10 ppm CCNE	11.34 <sup>a</sup>	9.02 <sup>a</sup>	1.270 <sup>b</sup>	0.764 <sup>a</sup>	0.395 <sup>a</sup>	0.064 <sup>c</sup>
<i>M. symbioticum</i> , + $\text{NH}_4\text{NO}_3$ at 50% + 10 ppm CCNE	12.74 <sup>a</sup>	10.24 <sup>a</sup>	1.528 <sup>a</sup>	0.765 <sup>a</sup>	0.166 <sup>b</sup>	0.109 <sup>b</sup>
<i>X. autotrophicus</i> + <i>M. symbioticum</i> $\text{NH}_4\text{NO}_3$ at 50% + 10 ppm CCNE	10.12 <sup>a</sup>	9.06 <sup>a</sup>	1.452 <sup>a</sup>	0.850 <sup>a</sup>	0.182 <sup>b</sup>	0.207 <sup>a</sup>

Table 2 and Figure 2 show the effect of *M. symbioticum* and *X. autotrophicus* on the growth of *P. vulgaris* with 50%  $\text{NH}_4\text{NO}_3$  and ppm

of CCNE. There it is observed that both *M. symbioticum* and *X. autotrophicus*, individually or in mixture, upon invading the seed of *P. vulgaris* fed with 50%  $\text{NH}_4\text{NO}_3$ , and then the root system of *P. vulgaris*, transformed the organic compounds derived from the beginning of germination into phytohormones that induced a greater and faster emergence of the *P. vulgaris* seeds (data not shown). Then, the colonization of the interior of the *P. vulgaris* roots allowed *M. symbioticum* and/or *X. autotrophicus* to transform compounds from root metabolism derived from photosynthesis, thus accelerating germination.<sup>29-31</sup> The rapid response of the *P. vulgaris* roots, combined with both physiological conditions, enhance the uptake optimization of 50%  $\text{NH}_4\text{NO}_3$  to avoid an excess of  $\text{NO}_3^-$ , which generally in the soil, causes water contamination and possible generation of  $\text{N}_2\text{O}$  due to the physicochemical conditions of the soil.<sup>5,32,33</sup> While the numerical values of *P. vulgaris* in the phenology: plant height, root length, and in the biomass: the aerial and root fresh weight as well as the aerial and root dry weight of *P. vulgaris* to seedling where in figure 3 shows the health of *P. vulgaris* when fed with  $\text{NH}_4\text{NO}_3$  at 50%, and inoculated with *M. symbioticum* and *X. autotrophicus* plus 10 ppm of CCNE.<sup>7-9</sup> In this regard, all the values for *P. vulgaris* fed with 50%  $\text{NH}_4\text{NO}_3$ , these endophytic genera of plant growth-promoting bacteria, and CCNE,<sup>34,35</sup> were statistically different compared to the values for *P. vulgaris* fed with 100%  $\text{NH}_4\text{NO}_3$  uninoculated with *M. symbioticum* and/or *X. autotrophicus*, and without applying CCNE.<sup>10,11</sup> This result made it clear that the dose normally recommended for *P. vulgaris* exceeds the uptake capacity of the root system, causing loss of organic matter in the soil, contamination of surface water and/or aquifers, and the possible release of  $\text{N}_2\text{O}$  from the unuptaken  $\text{NO}_3^-$ .<sup>4,5,36</sup>

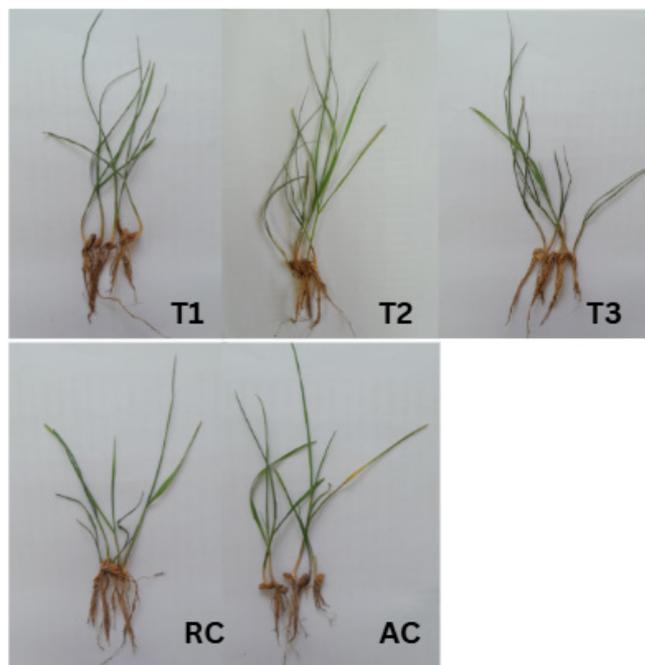


**Figure 2** Effect of *M. symbioticum* y *X. autotrophicus* on growth of *P. vulgaris*  $\text{NH}_4\text{NO}_3$  at 50% plus 10 ppm crude carbon nanoparticle extract (CCNE) at seedling stage.

AC: *P. vulgaris* + uninoculated + irrigated water

RC: *P. vulgaris* + uninoculated + fed mineral solution with  $\text{NH}_4\text{NO}_3$

T1: *P. vulgaris* + *M. symbioticum* +  $\text{NH}_4\text{NO}_3$ , 50% plus 10 ppm CCNE,



T2: *P. vulgaris* + *X. autotrophicus* +  $\text{NH}_4\text{NO}_3$  50% plus 10 ppm CCNE,

T3: *P. vulgaris* + *M. symbioticum* +  $\text{NH}_4\text{NO}_3$  50% plus 10 ppm CCNE.

**Figure 3** Effect of *M. symbioticum* and *X. autotrophicus* on growth of *T. aestivum* with  $\text{NH}_4\text{NO}_3$  at 50% plus 10 ppm crude carbon nanoparticles extract (CCNE).

AC: *T. aestivum* + uninoculated + water

RC: *T. aestivum* + uninoculated + fed mineral solution and  $\text{NH}_4\text{NO}_3$  at 100%

T1: *T. aestivum* + *M. symbioticum* + fed mineral solution and  $\text{NH}_4\text{NO}_3$  50% + 10 ppm CCNE,

T2: *T. aestivum* + *X. autotrophicus* + fed mineral solution and  $\text{NH}_4\text{NO}_3$  50% + 10 ppm CCNE,

T3: *T. aestivum* + *X. autotrophicus* + *M. symbioticum* + fed mineral solution and  $\text{NH}_4\text{NO}_3$  50% + 10 ppm CCNE.

**Table 3** Effect of *M. symbioticum* y *X. autotrophicus* on *T. aestivum* with  $\text{NH}_4\text{NO}_3$  at 50% plus 10 ppm crude carbon nanoparticles extract (CCNE) at seedling stage.

<i>Triticum aestivum</i> *	Plant height (cm)	Radical length (cm)	Fresh aerial weight (g)	Fresh radical weight (g)	Aerial dry weight (g)	Radical dry weight (g)
AC=absolute control uninoculated irrigated water	7.58 <sup>d</sup>	4.32 <sup>c</sup>	0.0204 <sup>d</sup>	0.0158 <sup>d</sup>	0.0102 <sup>c</sup>	0.0074 <sup>d</sup>
RC = relative control uninoculated fed with mineral solution + $\text{NH}_4\text{NO}_3$ at 100%,	8.48 <sup>c</sup>	3.82 <sup>d</sup>	0.0414 <sup>c</sup>	0.0310 <sup>c</sup>	0.0142 <sup>b</sup>	0.0086 <sup>d</sup>
<i>X. autotrophicus</i> , fed mineral solution + $\text{NH}_4\text{NO}_3$ at 50% + 10 ppm CCNE	10.58 <sup>b</sup>	5.64 <sup>b</sup>	0.0422 <sup>c</sup>	0.0482 <sup>b</sup>	0.0160 <sup>b</sup>	0.0290 <sup>a</sup>
<i>M. symbioticum</i> fed mineral solution + $\text{NH}_4\text{NO}_3$ at 50% + 10 ppm CCNE	15.82 <sup>a</sup>	5.52 <sup>b</sup>	0.1031 <sup>a</sup>	0.0497 <sup>a</sup>	0.0310 <sup>a</sup>	0.0102 <sup>c</sup>
<i>X. autotrophicus</i> + <i>M. symbioticum</i> fed mineral solution+ $\text{NH}_4\text{NO}_3$ at 50% + 10 ppm CCNE	11.46 <sup>b</sup>	7.1 <sup>a</sup>	0.0577 <sup>b</sup>	0.0329 <sup>c</sup>	0.0147 <sup>b</sup>	0.0158 <sup>b</sup>

Table 3 and Figure 3 show the effect of *M. symbioticum* and *X. autotrophicus* on the growth of *T. aestivum* with 50%  $\text{NH}_4\text{NO}_3$  and ppm of CCNE. In this case, it was demonstrated that both *M. symbioticum* and *X. autotrophicus*, separately or co-inoculated (T1-T3), were able to invade the *T. aestivum* seed fed with 50%  $\text{NH}_4\text{NO}_3$  and then colonize the root system of *T. aestivum*. *M. symbioticum* and *X. autotrophicus*, transformed the amino acids and sugars derived from the beginning of germination into phytohormones that increased the greater and faster emergence of *T. aestivum* seeds<sup>35</sup> (data not shown). Subsequently, *M. symbioticum* and/or *X. autotrophicus* colonized the roots of *T. aestivum*. *M. symbioticum* converted root sugars from photosynthesis, while the use of CCNE accelerated germination and the rapid mobilization of phytohormones that induced the growth of *T. aestivum* roots, increasing the contact surface in the soil to optimize the uptake of 50%  $\text{NH}_4\text{NO}_3$  as shown in Figure 3, where a higher root density was observed, which support the maximum uptake of  $\text{NH}_4\text{NO}_3$  to 50%.<sup>36-40</sup> Consequently, there were no  $\text{NO}_3^-$  remnants in the soil that cause accelerated loss of organic matter and generation of  $\text{N}_2\text{O}$  when the anaerobic heterotrophic microbiota in the soil uses  $\text{NO}_3^-$  as the final electron acceptor.<sup>4,5</sup> Therefore, in general, the numerical values of *T. aestivum* in phenology: plant height and root length and biomass: aerial and root fresh weight, as well as aerial and root dry weight; from germination to seedling stage (Figure 3) showed the enhanced growth of *T. aestivum* when fed with 50%  $\text{NH}_4\text{NO}_3$ , *M. symbioticum*, and *X. autotrophicus*, as well as with 10 ppm of CCNE.<sup>7-9</sup> Consequently, the numerical values were statistically different compared to similar values in *T. aestivum* fed with 100%  $\text{NH}_4\text{NO}_3$  or relative control (RC), uninoculated with *M. symbioticum* and/or *X. autotrophicus*, and without the use of CCNE.<sup>10,11</sup> This result made it clear that the 100%  $\text{NH}_4\text{NO}_3$  dose is greater than the uptake capacity of the *T. aestivum* root system, which in the soil, in addition to the loss of organic matter, contaminates surface water and/or aquifers as  $\text{N}_2\text{O}$  generation from the  $\text{NO}_3^-$  not uptaken.<sup>4,5,7,10,40</sup>

## Conclusion

It is concluded that the origin of the endophytic genera and species of plant growth-promoting bacteria *M. symbioticum* and *X. autotrophicus* did not influence their ability to recognize organic compounds in the seeds of *P. vulgaris* and *T. aestivum*. This is because both domesticated plants share the same type of photosynthesis, using organic compounds that are converted into phytohormones by *M. symbioticum* and/or *X. autotrophicus*. This establishes a positive

interaction in optimizing  $\text{NH}_4\text{NO}_3$  uptake to 50%. This process was accelerated and improved by the crude carbon nanoparticle extract, which favors  $\text{NH}_4\text{NO}_3$  optimization by improving the uptake capacity of the nitrogen fertilizer. This is especially true because *M. symbioticum* can move through the vascular system of both *P. vulgaris* and *T. aestivum*. Consequently, *M. symbioticum* and *X. autotrophicus* can be considered excellent option for optimizing the uptake of  $\text{NH}_4\text{NO}_3$ , 50% without risk of soil fertility loss, contamination of surface water or aquifers, or release of  $\text{N}_2\text{O}$  that causes global warming.

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## Conflicts of interest

The authors declare no conflicts of interest.

## References

1. Afzal I, Shinwari ZK, Sikandar S, et al. Plant beneficial endophytic bacteria: mechanisms, diversity, host range, and genetic determinants. *Microbiol. Res.* 2019;221:36–49.
2. Berg G, Erlacher A, Grube M. The edible plant microbiome: importance and health issues. In: *Principles of Plant-Microbe Interactions*. Springer; 2015.
3. Barrera SE, Sarango-Flóres SW, Montenegro-Gómez SP. The phyllosphere microbiome and its potential application in horticultural crops: A review. *Rev Colomb Cienc Hortíc.* 2019;13(3):384–396.
4. Cruz-Cárdenas C, Zelaya-Molina LX, Chávez-Díaz IF, et al. Microorganismos promotores de germinación en el cultivo de frijol. *Cienc Tecnol Agropecu Mex.* 2022;10(1):36–44.
5. González-Estrada A, Camacho Amador M. Emisión de gases de efecto invernadero de la fertilización nitrogenada en México. *Rev Mex Cienc Agríc.* 2017;8(8):1733–1745.

6. Eid AM, Fouda A, Abdel-Rahman MA, et al. Harnessing bacterial endophytes for promotion of plant growth and biotechnological application an overview. *Plants*. 2021;10(5):935.
7. De La Torre-Roche R, Hawthorne J, Deng Y, et al. Multiwalled carbon nanotubes and C60 fullerenes differentially impact the accumulation of weathered pesticides in four agricultural plants. *Env Sci Tec*. 2013;47(21):12539–12547.
8. Farooq MA, Ma W, Shen S, et al. Underlying biochemical and molecular mechanisms for seed germination. *Int J Mol Sci*. 2022;23(15):8502.
9. Fincheira P, Tortella G, Duran N, et al. Current applications of nanotechnology to develop plant growth inducer agents as an innovation strategy. *Cri Rev Bio*. 2020;40(1):15–30.
10. Liu H, Carvalhais LC, Crawford M, et al. Inner plant values: diversity, colonization and benefits from endophytic bacteria. *Front. Microbiol*. 2017;8:2552.
11. Firdous J, Mona R, Muhamad N. Endophytic bacteria and their potential application in agriculture: A review. *Indian Journal of Agricultural Research*. 2019;53(1).
12. Green PN. *Methylobacterium*. In: *Bergey's manual of systematics of archaea and bacteria*. Wiley, New York, USA. 2015. p. 1–8.
13. Ochsner AM, Sonntag F, Buchhaupt M, et al. *Methylobacterium extorquens*: methylotrophy and biotechnological applications. *Appl Microbiol Biotechnol*. 2015;99(2):517–534.
14. Jinal HN, Amaresan N, Sankaranarayanan A. et al. Herbaspirillum. In: Amaresan N, et al., editors. *Beneficial microbes in agro-ecology: bacteria and fungi*. Academic Press, New York, USA; 2019. p. 509–519.
15. Kour D, Kaur T, Devi R, et al. Biotechnological applications of beneficial microbiomes for evergreen agriculture and human health. In *New and Future Developments in Microbial Biotechnology and Bioengineering*. Elsevier.UK. 2020. p. 255–279.
16. Kour D, Rana KL, Yadav N, et al. Rhizospheric microbiomes: biodiversity, mechanisms of plant growth promotion, and biotechnological applications for sustainable agriculture. In: Kumar A, et al., editors. *Plant growth promoting rhizobacteria for agricultural sustainability: from theory to practices*. Springer. 2019. p. 9–65.
17. Khare E, Mishra J, Arora NK. Multifaceted interactions between endophytes and plant: developments and prospects. *Front. Microbiol*. 2018;9:2732
18. Majeed A, Abbasi MK, Hameed S, et al. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. *Front Microbiol*. 2015;6:198.
19. Matos AD, Gomes IC, Nietsche S, et al. Phosphate solubilization by endophytic bacteria isolated from banana trees. *Anais da Academia Brasileira de Ciências*. 2017;89(4):2945–2954.
20. Matteoli FP, Olivares FL, Venancio TM, et al. *Herbaspirillum*. In: Amaresan N, et al., eds. *Beneficial microbes in agro-ecology: bacteria and fungi*. Academic Press. 2020:493–508.
21. Mishra S, Bhattacharjee A, Sharma S. An ecological insight into the multifaceted world of plant-endophyte association. *Critical Reviews in Plant Sciences*. 2020;40(2):127–146.
22. Madhaiyan M, Poonguzhali S, Senthilkumar M, et al. *Methylobacterium gossipiicola* sp. nov., a pink pigmented, facultatively methylotrophic bacterium isolated from the cotton phyllosphere. *Int J Syst Evol Microbiol*. 2012;62(1):162–167.
23. Madhaiyan M, Alex THH, Ngoh ST, et al. Leaf-residing *Methylobacterium* species fix nitrogen and promote biomass and seed production in *Jatropha curcas*. *Biotechnol Biofuels*. 2015;8:222.
24. Mahanty T, Bhattacharjee S, Goswami M, et al. Biofertilizers: a potential approach for sustainable agriculture development. *Environ Sci Pollut Res Int*. 2017;24(4):3315–3335.
25. Norma oficial mexicana, que establece las especificaciones de fertilidad, salinidad y clasificación de suelos. Estudios, muestreo y análisis. Norma Oficial Mexicana NOM-021- RECNAT-2000. Diario Oficial de la Federación, 31 de diciembre de 2002.
26. Numan M, Bashir S, Khan Y, et al. Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: a review. *Microbiological research*. 2018;209:21–32.
27. Palberg D, Kisiala A, Jorge GL, et al. A survey of *Methylobacterium* species and strains reveals widespread production and varying profiles of cytokinin phytohormones. *BMC Microbiol*. 2022;22(1):49.
28. Priya M, Kumutha K, Senthilkumar M. Impact of bacterization of *Rhizobium* and *Methylobacterium radiotolerans* on germination and survivability in groundnut seed. *Int J Curr Microbiol App Sci*. 2019;2;8(8):394–405.
29. Pattnaik S, Rajkumari J, Paramanandham P, et al. Indole acetic acid production and growth-promoting activity of *Methylobacterium extorquens* MP<sub>1</sub> and *Methylobacterium zatmanii* MS<sub>4</sub> in tomato. *Int J Veg Sci*. 2017;23(4):321–330.
30. Srivastava A, Dixit R, Chand N, et al. Overview of methylotrophic microorganisms in agriculture. *Bio Sci Res Bull*. 2022;238:65–71.
31. Ramakrishna W, Yadav R, Li K. Plant growth promoting bacteria in agriculture: two sides of a coin. *Applied Soil Ecology*. 2019;138:10–18.
32. Rana KL, Kour D, Kaur T, et al. Endophytic microbes: biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. *Antonie Van Leeuwenhoek*. 2020;113(8):1075–1107.
33. Schlaeppi K, Bulgarelli D. The plant microbiome at work. *Mol Plant Microbe Interact*. 2015;28 (3):212–217.
34. Singh M, Singh D, Gupta A, et al. *Plant growth promoting Rhizobacteria: application in biofertilizers and biocontrol of phytopathogens*. In: PGPR Amelioration in Sustainable Agriculture. Woodhead Publishing, 2019. p. 41–66.
35. Ton A. Advantages of grain legume-cereal intercropping in sustainable agriculture. *Turk J Agric Food Sci Technol*. 2021;9(8):1560–1566.
36. Vogel BC, Bai Y, Vorholt JA. The plant microbiota: systems-level insights and perspectives. *Annu Rev Genet*. 2016;50:211–234.
37. Yadav AN, Kumar R, Kumar S, et al. Beneficial microbiomes: biodiversity and potential biotechnological applications for sustainable agriculture and human health. *J Appl Biol Biotechnol*. 2017;5(6):45–57.
38. Vera RT, García AJB, Álvarez FJC, et al. Application and effectiveness of *Methylobacterium symbioticum* as a biological inoculant in maize and strawberry crops. *Folia Microbiol (Praha)*. 2024;69(1):121–131.
39. Yadav AN, Kumar V, Prasad R, et al. Microbiome in crops: diversity, distribution and potential role in crop improvements. In: Prasad R, Gill SS, Tuteja N, eds. *Crop improvement through microbial biotechnology*. Elsevier; 2018. p. 305–332.
40. Yadav AN, Verma P, Kour D, et al. Plant microbiomes and its beneficial multifunctional plant growth promoting attributes. *Int J Environ Sci Nat Resour*. 2017;3(1):1–8.