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Bacillus thuringiensis and Micromonospora echinospora on growth of Phaseolus vulgaris

Abstract

The well-known bacteria genus called as *Bacillus thuringiensis* (*Bt*) is recognized for being used in the biological control of insect pests in agriculture. However, there is little information on the effect of *Bt* as a plant growth promoter and in interaction with *Micromonospora echinopora*, which, being an endophyte, could improve the response of *Phaseolus vulgaris* with NH_4NO_3 atreduced at 30%of common recommended without risk of affecting plant health or yield.The objective of this work was to analyze the response of *P. vulgaris* to *Bt* and *M. echinospora* with NH_4NO_3 at 30% of recommended dose. The experiment was conducted under a randomized block design, using the response variables: germination percentage, phenology and biomass. The experimental data were analyzed by ANOVA/Tukey HSD ($P<0.05$). In the results, it was found that *B.thuringiensis* induced in *P. vulgaris* a yield of 3.2Ton/Ha, while with *M. echinospora* 3.6Ton/Ha was reached, with NF reduced at 30%, while it was shown that *B.thuringiensis* improved the yield with *M. echinospora* with 4.0Ton /Ha, values with statistical difference compared to the 2.8 Ton/Ha of *P. vulgaris* without *B.thuringiensis* or *M. echinospora* used as relative control with 100% of the NF.Concluded that *B. thuringiensis* is not only useful in biological control but that it can be an excellent growth promoter that can be improved with *M. echinospora*, another option for the sustainable production of *P. vulgaris*.

Keywords: legume, soil, hyperfertilization, beneficial endophytic bacteria, plant health.

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Introduction

The healthy growth of *Phaseolus vulgaris* (bean) requires nitrogen fertilizer (NF) commonly as a NH_4NO_3, which at high doses causes loss of soil productiveness due to rapid mineralization of organic matter,[1-3] An natural and ecological alternative solution to optimization of NF in *P. vulgaris* from seed inoculation with *Bacillus thuringiensis* and *Micromonospora echinospora*, genera and species of plant growth-promoting endophytic bacteria (PGPEB)which convert exudates from the seed spermosphere and inside the roots into plant growth promoting substancesor phytohormones that accelerate germination and induce a denser root system for the absorption and optimization of the NF applied at doses lower than those recommended, without affecting healthy plant growth.[4-7] Generally, for *P. vulgaris,* inoculants based on *Rhizobium etli* are recommended, a symbiote of this class of legumes, which is dependent on the variety of *P. vulgaris* and/or the soil. They may not be infective and effective in facilitating healthy growth at doses of NF for sustainable production. Based in some reports that showed that other bacteria could be apply in *P. vulgaris* and other legumescould be inoculated with *B. megaterium*[8] or with actinomycetes like*Streptomyces flavoviridis* a.[9] Therefor the objective of this research was to analyze the response of *P. vulgaris* to *B. thuringiensis* and *M. echinospora* with NH_4NO_3 at 30%.

Materials and Methods

This research was carried out in the greenhouse of the Environmental Microbiology laboratory, Instituto de Investigaciones Químico-Biológicas (IIQB) of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Morelia, Mich, Mexico, with the average microclimatic conditions were: temperature of 23.2°C, luminosity of 450µmol-$m^{-2}s^{-1}$ and 67% relative humidity. In this trial, a non-sterile soil collected from the municipality of Salvador Escalante Michoacán, Mexico, with a silty clay loam texture with 10.44% organic matter and pH of 5.75 moderately acidic according to NOM-021-RECNAT-2000 was used.[10] 20 mesh and solarized at 70°C/48h to minimize pests and diseases, then 1.0kg of soil was placed in the top container of the Leonard jar (Figure 1), while NF in a mineral solution or water in the reservoir at the bottom, *P. vulgaris* seeds were disinfected with 5% NaClO/5min and washed 5 times with sterile drinking water, then disinfected in 70% alcohol/ 5min and washed 5 times with sterile drinking water, then *B. thuringiensis* isolated from soil rich in organic matter was activated in nutrient broth with the following chemical composition (g/L): casein peptone 5; yeast extract 3; distilled water, adjusted the culture medium to pH 6.8±0.2; with the antifungal Tecto®60 (Syngenta), while *M. echinospora* isolated from nodules of *M. sativa* was propagated on avocado pit agar with the following chemical composition (g/L): avocado pit 10.0, casein peptone 5.0, yeast extract 1.3, K_2HPO_4 0.17, KH_2PO_4 2.61, $MgSO_4$ 1.5, NaCl 0. 9, $CuSO_4$ 0.05, bromothymol blue 10% (w/v) 10ppm, trace element solution 1.0mL, detergent solution 2.5mL at 10%, antifungal Tecto®60 1.0mL at 10%, 1.0mL, bacteriological agar 18.0, at pH 7.5, both bacterial genera were incubated at 30°C/72h. Then for every 10 *P. vulgaris* seeds, 1.0mL of *B. thuringiensis* was used at a density of $4.6x10^6$CFU/mL and *M. echinospora* at a density of $3.5x10^6$ propagule forming units/mL. Table 1 describes the experimental design with 2 controls and 6treatments with 6 replicates: *P. vulgaris* irrigated with water or absolute control (AC); *P. vulgaris* fed with NFat 100%or relative control (RC); *P. vulgaris* with *B. thuringiensis* and/or *M. echinospora* with 30% reduced NF in a mineral solution with the following chemical composition (g/L): NH_4NO_3 10.0, K_2HPO_4 2.5, KH_2PO_4 2.0, $MgSO_4$ 1.0, NaCl 0.1, $CaCl_2$ 0.1, trace $FeSO_4$, trace element solution 10.0mL, adjusted to pH 6.5-6.8. The NF was applied every third day at 80% field capacity.[11] The response variables were: percentage (%) of germination and days of emergence; phenology: plant height (PH), root length (RL); biomass: aerial fresh weight (AFW) and root fresh weight (AFW), and aerial dry weight (ADW) and root dry weight (RDW) at seedling, flowering and physiological maturity stages and yield, the experimental data were validated with the statistical program ANOVA/Tukey HSD $P<0.05$ with Statgraphics Centurion.[13]

Results

In Table 2 shows the percentage and days of emergence of *P. vulgaris* seeds with *B. thuringiensis* and/or *M. echinospora* and

NH_4NO_3 at 30%, emerged 3days after sowing, or 94% germination a numerical value stadistically different compared to 5days of emergence or 83% of *P. vulgaris* without*B. thuringiensis/M. echinospora* and $NH_4NO_3$100% or relative control (RC).

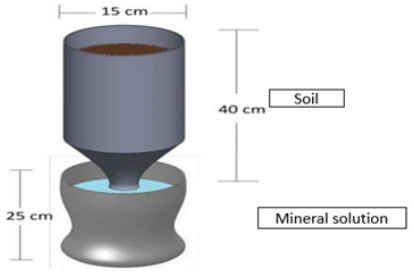

Figure 1 Leonard's jar design.[12]

Table 1 Experimental design to analyze the growth of *Phaseolus vulgaris* to *Bacillus thuringiensis* and *Micromonospora echinospora* with NH_4NO_3 reduced at 30%

Phaseolus Vulgaris	**Water**	**NH_4NO_3**	***Bacillus thuringiensis***	***Micro monspora echino spora***
Absolute control (AC)	+	-	-	-
Relative control (RC)	-	100%	-	-
Treatment 1	-	30%	+	-
Treatment 2	-	30%	-	+
Treatment 3	-	30%	+	+

*Number of repetitions = 6; (+) added; (-) not added.

Table 2 Percentage and days to emergence of *Phaseolus vulgaris* with *Bacillus thuringiensis* and *Micromonospora echinospora* andNH_4NO_3 reduced at 30%

Treatment/*P. vulgaris**	**Percent (%) germination**	**Days emergence**
Irrigated with water (AC)	77^{d**}	$**5^{b}$
with NH_4NO_3 at 100% (RC)	83^{b}	5^{b}
B. thuringiensis +NH_4NO_3at 30%	94^{a}	3^{a}
M. echinospora + NH_4NO_3at 30%	91^{a}	3^{a}
B. thuringiensis/M. echinospora +$NH_4NO_3$30%	94^{a}	3^{a}

*n=6, ** Different letters indicate statistical difference by ANOVA/Tukey (P>0.05).

In Figure 2 shows that the seed of *P. vulgaris*with NH_4NO_3 at 30% emerged 5 days after sowing, due to*B. thuringiensis /M echinospora*by phytohormons from organic compound from the seed induced the rapid formation of a stem-and-root primordium compared to the seed of *P. vulgaris* without *B. thuringiensis /M echinospora*and NH_4NO_3at 100% (RC).

In Table 3 shows at the seedling level stage the positive response of *P. vulgaris* to *M. echinospora* with NF at 30%, which registered 42.33cm of PH and 16.16cm of RL, as well as 2.82g of AFW, 2.53g of RFW, 0.31g of ADW and 0. 11g of RDW, numerical values with statistical difference compared to phenology data: 30.83cm of PH and 14.33cm of RL; and those of biomass: 1.08g of AFW, 0.76g of RFW, 0.15g of ADW and 0.04g of RDW of *P. vulgaris* without *B. thuringiensis* and *M. echinospora* with NH_4NO_3 at 100% (RC).

In Figure 3 shows the growth of *P. vulgaris* with*B. thuringiensis* and *M. echinospora*and NH_4NO_3 at 30%, with increase in stem height and agreat density of the root system, compared to *P. vulgaris* without *B. thuringiensis* and *M. echinospora* with t at 100% (RC), since the stem had a smaller diameter and the root system was less dense.

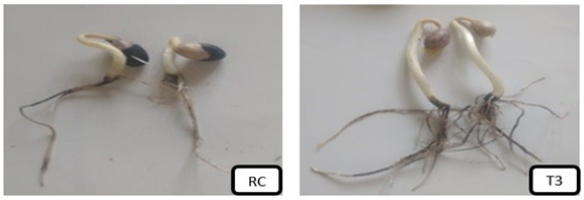

Figure 2 Germination of *Phaseolus vulgaris*with *Bacillus thuringiensis* and *Micromonospora Echinospora* with NH_4NO_3 reduced at 30%.

Relative control (RC) = *P. vulgaris* without *B. thuringiensis/M. echinospora* fed withNH_4NO_3 at 100%.

Treatment (T3) =P. vulgaris with B. thuringiensis/M echinospora NH_4NO at 30%.

Figure 3 Growth of *Phaseolus vulgaris* with*Bacillus thuringiensis* and/or *Micromonospora chinospora*at seedling level andNH_4NO_3reduced at 30%.

Absolute control (AC) = *P. vulgaris* without*B. thuringiensis/M. echinospora* irrigated with water;

Relative control (RC) = *P. vulgaris* without *B. thuringiensis/M. echinospora* fed with NH_4NO_3 at 100% ;

Treatment (T) 1= *P. vulgaris* with*B. thuringiensis* + NH_4NO_3 at 30%;

T2= *P. vulgaris* withM. e*chinospora* +NH_4NO_3 at 30%;

T3= P. vulgaris withB. thuringiensis/M. echinospora + NH4NO3at 30%.

Table 3 Growth of *Phaseolus vulgaris* to *Bacillus thuringiensis* and *Micromonospora echinospora* at seedling levelwith NH_4NO_3 reduced at30%

Treatment/*P. vulgaris**	**Plant height (cm)**	**Root length (cm)**	**Fresh weight (g)**		**Dry weight (g)**	
			Aerial	**Root**	**Aerial**	**Root**
Irrigated with water (AC)	18.5^{d**}	9.16^{c}	0.85^{b}	0.43^{c}	$0.08^{d}c$	0.02^{d}
+ NH_4NO_3at 100% (RC)	30.83^{c}	14.33^{b}	1.08^{b}	0.76^{b}	0.15^{bb}	0.04^{c}
B. thuringiensis +NH_4NO_3at 30%	36.5^{b}	16.16^{a}	2.69^{a}	2.26^{a}	0.22^{ab}	0.08^{b}
M. echinospora + NH_4NO_3 at30%	42.33^{a}	16.16^{a}	2.82^{a}	2.35^{a}	0.31^{aa}	0.11^{a}
B. thuringiensis/M. echinospora +NH_4NO_3at 30%	37.33^{a}	15.66^{a}	2.13^{a}	2.05^{a}	0.25^{aa}	0.11^{a}

*n=6, ** Different letters indicate statistical difference by ANOVA/Tukey (P>0.05).

In Table 4 shows the response of *P. vulgaris* to *B. thuringiensis* and *M. echinospora* at flowering level with the NF at 30% reduced dose, which registered 71.33cm of PH and 26.33cm of RL, 5.67g of AFW, 5.64g of RFW, 1.61g of ADW and 0. 32g of RWD, numerical values with statistical difference compared to 59.9cm of PH, 15.5cm of RL, 3.54g of AFW, 0.78g of RFW, 0.32g of ADW and 0.07 g of RDW of *P. vulgaris* without *B. thuringiensis* or *M. echinospora* with NH_4NO_3 at 100% or RC.

In Figure 4, showed *P. vulgaris* at flowering level with *B. thuringiensis* and *M. echinospora* with NH_4NO_3at 30%, recorded an increase in stem thickness, greater size and number of leaves and with abundant root area system, compared to *P. vulgaris* without *B. thuringiensis* and *M. echinospora* with NH_4NO_3 at 100% or RC which had small leaves, less stem thickness and a smaller root system.

Table 4 Growth of *Phaseolus vulgaris* to *Bacillus thuringiensis* and *Micromonospora echinospora* at flowering level andNH_4NO_3reducedat 30%

Treatment/*P. vulgaris**	Plant height (cm)	Root length (cm)	Fresh weight (g)		Dry weight (g)	
			Aerial	Root	Aerial	Root
Irrigated with water (AC)	52.0^{c**}	12.83^{c}	1.80^{d}	0.40^{b}	0.26^{c}	0.04^{c}
with nitrogen fertilizer at 100% (RC)	59.9^{bc}	15.5^{c}	3.54^{c}	0.78^{b}	0.32^{c}	0.07^{c}
B. thuringiensis + NF at 30%	62.16^{a}	18.33^{b}	4.90^{a}	4.46^{a}	0.84^{b}	0.17^{b}
M. echinospora + NF at30%	63.66^{a}	24.66^{a}	5.54^{a}	5.36^{a}	1.15^{a}	0.25^{a}
B. thuringiensis/*M. echinospora* + NF at 30%	71.33^{a}	26.33^{a}	5.67^{a}	5.64^{a}	1.61^{a}	0.32^{a}

*n=6, ** Different letters indicate statistical difference by ANOVA/Tukey (P>0.05).

Figure 4 Growth of *Phaseolus vulgaris* to *Bacillus thuringiensis* and *Micromonospora echinospora* at flowering level andNH_4NO_3reduced at 30%.

Absolute control (AC) = *P. vulgaris* without *B. thuringiensis*/*M. echinospora* irrigated with water;

Relative control (RC) = *P. vulgaris* without *B. thuringiensis*/*M. echinospora*fed with NH_4NO_3at 100% ;

Treatment (T1)=*P. vulgaris* with *B. thuringiensis*with NH_4NO_3 at 30%;

T2= *P. vulgaris* with *M. echinospora* + NH_4NO_3at 30 %,

T3= *P. vulgaris* with *B. thuringiensis*/*M. echinospora* +NH_4NO_3at 30%.

In Table 5 shows at the physiological maturity level the response of *P. vulgaris* to *B. thuringiensis* and *M. echinospora* with NH_4NO_3 reduced to 30%, where it registered 125.5cm of PH and 8.83cm of RL, as well as 8.99g of AFW, 8.35g of RFW, 3.83g of ADW and 0.33g of RDW, these numerical values had statistical difference in relation to: 91.16cm of PH, 23.33cm of RL, 4.42g of AFW, 3.34g of RFW, 1.73g of ADW and 0.05g of RDW of *P. vulgaris* without *B. thuringiensis* and *M. echinospora* with NH_4NO_3 at 100% (RC).

In Figure 5 shows the growth of*P. vulgaris* at physiological maturity 1 to *B. thuringiensis* and *M. echinospora* with NH_4NO_3 at 30%; where a larger stem diameter was observed along with an intense green coloration, larger spike size and increased root system, compared to *P. vulgaris* without *B. thuringiensis* and *M. echinospora* with NH_4NO_3 at 100% or relative control that had a smaller spike and less density in the root system.

In Table 6 shows the response of *P. vulgaris* in biomass and fruit yield to *B. thuringiensis* and *M. echinospora* with NH_4NO_3 at 30% reduced dose, which registered 7.70g of fresh fruit weightand 40g of total fruit weight with 4.0Ton/ha, these values had statistical difference in relation to the 3.15g of fresh fruit weight, 28g of total fruit weight and 2.8Ton/ha yield of *P. vulgaris* without *B. thuringiensis* and *M. echinospora* with NH_4NO_3 at 100% (RC).

Table 5 Growth of Phaseolus vulgaris at physiological maturity level to *Bacillus thuringiensis* and *Micromonospora echinospora*and NH_4NO_3reducedat 30%

Treatment/*P. vulgaris**	Plant height (cm)	Root length (cm)	Fresh weight (g)		Dry weight (g)	
			Aerial	Root	Aerial	Root
Irrigated with water (AC)	68.83^{b**}	15.33^{c}	3.35^{b}	2.72^{c}	0.93^{d}	0.03^{d}
withNH_4NO_3at 100% (RC)	**91.16^{b}**	**23.33^{b}**	**4.92^{b}**	**3.34^{c}**	**1.73^{c}**	**0.05^{d}**
B. thuringiensis +NH_4NO_3at 30%	**98.16^{a}**	**26.5^{a}**	**7.55^{a}**	**6.06^{b}**	**2.63^{b}**	**0.23^{b}**
M. echinospora + NH_4NO_3 at 30%	**117^{a}**	**27^{a}**	**7.90^{a}**	**7.08^{a}**	**3.80^{a}**	**0.28^{a}**
B. thuringiensis/*M. echinospora* + NH_4NO_3 at 30%	**125.5^{a}**	**28.83^{a}**	**8.99^{a}**	**8.35^{a}**	**3.83^{a}**	**0.33^{a}**

*n=6, ** Different letters indicate statistical difference by ANOVA/Tukey (P>0.05).

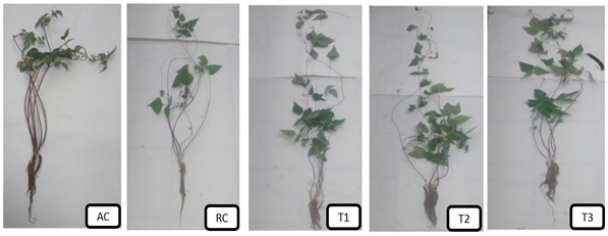

Figure 5 Growth of *Phaseolus vulgaris* at physiological maturity level with*Bacillus thuringiensis*and *Micromonospora echinospora*andNH_4NO_3reduced at 30%.

Absolute control (AC) = *P. vulgaris* without *B. thuringiensis*/*M. echinospora* irrigated with water;

Relative control (RC) = *P. vulgaris* without *B. thuringiensis*/*M. echinospora*+ NH_4NO_3at 100%

Treatment (T1) =*P. vulgaris* with*B. thuringiensis* + NH_4NO_3 30%;

T2= *P. vulgaris* with *M. echinospora* + NH_4NO_3 30%;

T3= *P. vulgaris* with *B. thuringiensis*/*M. echinospora* +NH_4NO_3at 30%.

Table 6 Yield elements of *Phaseolus vulgaris* with *Bacillus thuringiensis* and *Micromonospora echinospora*andNH_4NO_3 reduced at 30%

Treatment/*P. vulgaris**	Fresh weight of fruit (g)	Total weight of fruit weight (g)	Yield (Ton/ ha)
Irrigated with water (AC)	1.55^{c**}	20^{c}	0.2^{d}
withNH_4NO_3at 100% (RC)	3.15^{b}	28^{b}	2.8^{b}
B. thuringiensis +NH_4NO_3at30%	5.48^{a}	32^{b}	3.2^{a}
M. echinospora + NH_4NO_3 at30%	6.09^{a}	36^{a}	3.6^{a}
B. thuringiensis/*M. echinospora* +NH_4NO_3 at 30%	7.70^{a}	40^{a}	4.0^{a}

*n=6, ** Different letters indicate statistical difference by ANOVA/Tukey (P>0.05).

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In Figure 6 shows the beneficial effect of *B. thuringiensis* and *M. echinospora* on the pods of *P. vulgaris* with the NH_4NO_3 at 30%, where an increase in size, intense green coloration and higher number of seeds were observed compared to the pods of *P. vulgaris* without inoculation with *B. thuringiensis* and *M. echinospora* with the NH_4NO_3 at 100% or RC, which are smaller and with fewer seeds.

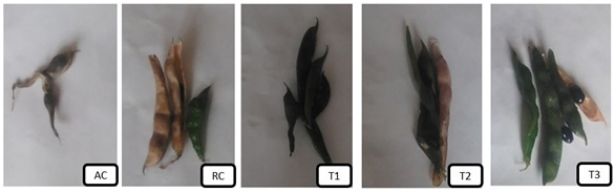

Figure 6 Elements of yield of *Phaseolus vulgaris*with *Bacillus thuringiensis* and *Micromonospora echinospora*andNH_4NO_3reduced at 30%.

Absolute control (AC) = *P.vulgaris* without *B.thuringiensis*/*M.echinospora*irrigated with water;

Relative control (RC) = *P. vulgaris* without *B. thuringiensis*/*M. echinospora* fed with NH_4NO_3 at 100%

Treatment (T1) =*P. vulgaris* with *B. thuringiensis*+ NH_4NO_3 at 30%;

T2= *P. vulgaris* with*M. echinospora* + NH_4NO_3 at 30%;

T3= P. vulgaris withB. thuringiensis/M. echinospora + NH_4NO_3 at 30%.

Discussion

The data presented in Table 2 support the response of *P. vulgaris* seed to *B. thuringiensis* and *M. echinospora*with NH_4NO_3at 30%, which fast emergence time of *P. vulgaris*seeds which were releasing organic compounds that *B. thuringiensis* and *M. echinospora* transformed into phytohormons that accelerated the emergence of root and seedling primordium.14-1 This fact that is confirmed in Figure 2, where *B. thuringiensis* and *M. echinospora* on *P. vulgaris* at the fifth day, after sowing showed the largest size of stem and root primordium, compared to *P. vulgaris* without *B. thuringiensis* and *M. echinospora*and NH_4NO_3 at 100%.

In Table 3, the numerical data of phenology and biomass of *P. vulgaris* with *M. echinospora* with NH_4NO_3 30% at seedling stage shows that *B. thuringiensis* and *M. echinospora* converted some organic compounds from photosynthesis into phytohormons inducing karyokinesisto increasemore density of root hairs to maximize NH_4NO_3 uptake at 30%.[17,18] As it's been reported by Gopalakrishnan et al.,[19] with *C. arietinum* to *Streptomyces* sp at NH_4NO_3 level of soil. Figure 3 demostratethat*M. echinospora* on *P. vulgaris*by phytohormones enhanced stem diameter and root density of *P. vulgaris* compared to *P. vulgaris* without *B. thuringiensis* and *M. echinospora* had lower stem diameter and root density, indicated that NH_4NO_3 at 100% shows that was not efficiently taken up by root of *P. vulgaris.*

In Table 4, are show values of phenology and biomass of *P. vulgaris* with *B. thuringiensis* and *M. echinospora* with NH_4NO_3 at 30% at flowering level, suggesting that which converted photosynthesis metabolites into phytohormons for *P. vulgaris* to reach the highest concentration in the apical zone, to improve and rapid stem and leaf growth[20,21] as is observed in Figure 4. The same way as Pérez-Fernández & Alexander,[22] in *C. arietinum*with *B. megaterium* that increased RDW by enhancing uptake of NH_4NO_3.

In Table 5, shows the growth of *P. vulgaris* in terms of the phenology and biomass at physiological maturity due to *B. thuringiensis* and *M. echinospora* with $NH_4NO_3$30%, its reported that organic compounds from photosynthesis transformed into phytohormones that stimulated the generation of vegetative axillary buds that induced root elongation,[22-24] in that sense the root systems were able to exploration soil to uptake and optimized NH_4NO_3 reduced at 30%, even that with healthy plant growth, compared to *P. vulgaris* without *B. thuringiensis* and *M. echinospora* fed with NH_4NO_3 at 100% (RC), the root system grew less as its observed in Figure 5, indicating that the recommended dose was not uptake.

In Table 6, the beneficial effect of *B. thuringiensis* and *M. echinospora* on the biomass and pod yield of *P. vulgaris* with the dose reduced NF to 30%, which support both PGPEB maintained the conversion of photosynthesis derived carbon compounds into phytohormones:¸¿ which at higher concentration stimulated the maturation of *P. vulgaris* pods despite the reduction of NF by up to 30%,[6,25] thus *P. vulgaris* had healthy growth by enhancing root system formation in the soil for maximum absorption and optimization of the NF applied at 30%.[26-28] The above is confirmed by what is shown in Figure 6, where the positive effect of *B. thuringiensis* and *M. echinospora* on *P. vulgaris* pods compared to those of RC is observed.

Conclusion

It was demonstrated that *B. thuringiensis* and *M. echinospora*, however being different genus and species endophytic bacteria are to improve germination and colonize the interior of the roots of *¨P. vulgaris;* to increased root uptake of the reduced NF by up to 30%, without compromising the healthy growth of *P. vulgaris*. In that sense *B. thuringiensis* and *M. echinospora* endophytes of wild plants of the *L. leucocephala* and *M. sativa* types are an option for sustainable production of *P. vulgaris* preventing soil lost productivity and pollution due to hyper fertilization**.**

Author Contributions

Conceptualization JMSY and AVM; data curation, JMSY, JLIC and AVM; writing—original draft preparation, JMSY and JLIC; writing—review and editing, JMSY and JLIC; Final version JMSY. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declared no have conflict interest for the study.

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