Rev. Int. Contam. Ambie. 40 (Ambiente y Bioenergía) 13-25, 2024
https://doi.org/10.20937/RICA.55011

PRODUCTION OF VEGETABLES WITH BIOFERTILIZERS BASED ON NATIVE MICROORGANISMS: A CASE STUDY IN TIXTLA, GUERRERO, MEXICO

Producción de hortalizas con biofertilizantes a base de microorganismos locales: estudio de caso en Tixtla, Guerrero, México

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(Received: June 2023; accepted: September 2023)

Key words: crop management, soil conservation, sustainable agriculture.

ABSTRACT

In agriculture, the use of fertilizers has increased due to the high demand for food by humanity, causing negative impacts because of high production costs and soil contamination. We evaluated five biofertilizers based on native microorganisms promoters of plant growth (NMPPG) as an alternative to the fertilizers used by small vegetable producers. The evaluation was carried out with three types of vegetables (lettuce, celery and epazote) in the Juan Berbera Catalán Irrigation Unit made up by producers from the community of Tixtla, Guerrero. The results show that *Rhizobium* sp. R01 (Tx3), *Azotobacter* sp. C3 (Tx1) and *Bacillus licheniformis* M2-7 (Tx5) used as biofertilizers promote germination and considerably increase fresh weight in these crops, and *Azospirillum* sp. M9 (Tx2) and *Trichoderma* sp. ABC1 (Tx4) present significant differences with the negative and chemical control. The yield of lettuce, celery and epazote was favored using biofertilizers, which can be considered a good alternative to apply to these crops, likewise, the price of biofertilizers (5 liters per Tx) compared to the fertilizer DAP-18-46-00 (50 kg) used by the same producers is $1,200.00 vs. $2,000.00 MXN per hectare produced respectively. In conclusion, the biofertilizers used herein represent a feasible alternative for farmers without disregarding the need for technical specialists and producers to identify optimal conditions that support the sustained application and management of biofertilizers.

Palabras clave: agricultura sostenible, conservación del suelo, gestión de cultivos.

RESUMEN

En la agricultura, el uso de fertilizantes se ha incrementado por la alta demanda de alimentos por la humanidad, provocando impactos negativos por los altos costos de producción y la contaminación del suelo. Evaluamos cinco biofertilizantes a base de microorganismos promotores de crecimiento vegetal nativos (MPCVN) como alternativa a los fertilizantes usados por los pequeños productores de hortalizas. La evaluación fue con tres tipos de hortalizas (lechuga, apio y epazote) en la Unidad de Riego Juan Berbera Catalán conformada por productores de la comunidad de Tixtla, Guerrero. Los resultados demuestran que *Rhizobium* sp. R01 (Tx3), *Azotobacter* sp. C2 (Tx1) y *Bacillus licheniformis M2-7* (Tx5) utilizadas como biofertilizantes, promueven la germinación y aumentan considerablemente el peso fresco en estos cultivos, mientras que *Azospirillum* sp. M9 (Tx2) y *Trichoderma* sp. ABC1 (Tx4) presentan diferencias significativas con el testigo negativo y químico. El rendimiento de la lechuga, apio y epazote se vio favorecido por el uso de los biofertilizantes lo que puede considerarse como una buena alternativa para aplicarse a estos cultivos, así mismo, el precio de los biofertilizantes (5 litros por Tx) en comparación con el fertilizante DAP-18-46-00 (50 kg) usado por los mismos productores es de $1200.00 vs $2,000.00 MN por hectárea producida respectivamente. Se puede concluir que los biofertilizantes aquí utilizados representan una alternativa viable para los agricultores y que resulta necesario que los técnicos especialistas y los productores identifiquen las condiciones óptimas que apoyen la aplicación y el manejo sostenidos de los biofertilizantes.

INTRODUCTION

Fertilizers have been used excessively in agriculture, providing nutrients to crops to produce food (ONUAA 2017). The problems associated with their application have led to the search for new alternatives to reduce its excessive use, being one of them the use of native microorganisms promoters of plant growth (NMPPG) as biofertilizers. The NMPPG have been reported to be beneficial to different vegetable crops, by optimizing root development, strengthening the response against diseases, protecting against pests, increasing production yields, raising quality, reducing costs, and preventing soil degradation (Bagyalakshmi et al. 2017, Bolaños et al. 2021, Martínez et al. 2020, Orbe et al. 2022).

According to Betancourt and Tizapa (2022), in the municipality of Tixtla, in the state of Guerrero, the inhabitants present health problems caused by the ingestion of water and food contaminated with agrochemicals. Besides, agricultural producers are still looking for alternatives to reduce the use of fertilizers due to rising prices and the environmental problems (soil erosion, contamination of aquifers, loss of microbial diversity, etc.) that have been detected because of the prolonged use of such chemicals. There are about 50 small producers in this municipality; most of them produce flowers and vegetables, mainly lettuce, radishes, cabbage, coriander, corn, among others and consider important the use of alternatives such as the application of biofertilizers made with NMPPG (Betancourt and Tizapa 2022) to maintain the adequate performance and quality of their agricultural products. This allows for a better economic income, and care for the environment and the human health (Martínez et al. 2020).

Therefore, this work aimed to evaluate the effectiveness of five biofertilizers based on native microorganisms promoting plant growth (NMPPG) as a sustainable alternative to chemical fertilizers used by small-scale vegetable producers. Specifically, the study aims to determine the impact of these biofertilizers on the germination, fresh weight increase, and yield of lettuce, celery, and epazote crops within the Juan Berbera Catalán Irrigation Unit, comprised of producers from the community of Tixtla, Guerrero. Additionally, the study intends to compare production costs and assess the environmental and human health benefits of using biofertilizers over traditional chemical fertilizers.

MATERIALS AND METHODS

Study area and contact with farmers

This study was carried out in the Juan Berbera Catalan Irrigation Unit, located in the community of Tixtla in Guerrero. A representative group of 8 farmers was contacted to discuss the need to apply alternatives to the use of fertilizers. Subsequently, a meeting was held with 50 producers from the Juan Catalan Berbera Irrigation Unit to demonstrate that the use of biofertilizers in three vegetable crops gave better cost alternatives, less damage to their health and was more environmentally friendly, and it could be implemented in future productions.

The installation of the demonstration plot where lettuce, celery and epazote were grown was by agreement with Mr. Alejandro Alcaraz Mendoza, president of the Irrigation Unit. The agreement established that the small producers would support the installation of the demonstration plot to carry out the experiment, and once completed, the final production would be made available to them (**Fig. 1**).

Treatment design

These treatments were applied to lettuce (*Lactuca sativa* var. longifolia), epazote (*Dysphania ambrosioides* L.) and celery (*Apium graveolens* var. secalinum). The treatments were: Tx1 (*Azotobacter* sp. C3), Tx2 (*Azospirillum* sp. M9), Tx3 (*Rhizobium* sp. R01), Tx4 (*Trichoderma* sp. ABC1), Tx5 (*Bacillus licheniformis* M2-7), Tx6 (water) and Tx7 (DAP-18-46-00).

Random complete block design

For each vegetable, the design of the plots was randomized complete blocks as described by Little and Jackson (2008). They were placed with 4 repetitions of 25 plants for a total of 100 plants per treatment, as shown in **fig. 2**.

Installation of the demonstration plot

To obtain lettuce, celery and epazote seedlings, the seeds obtained by the producers were planted in a nursery on 2 m² in their soils. The installation of the demonstration plot was established following the methodology described by the ONUAA (2015) in its soil improvement manual. The demonstrative plot was established in dimensions of 7 × 20 m. In addition, plants were added at the ends of the rows to rule out the border effect, but they were not included in the analysis of variables.

Microbial cultures

The NMPPG were activated in tubes with nutrient broth at 30 °C for 24 h, and then 10 mL of each strain were placed in 100 mL of sterile nutrient broth adjusting the optical density (OD) from 0.08 to 0.1 nm (nanometers), equivalent to 1×10^8 CFU/ml estimated with a spectrophotometer (Orbe et al. 2022). *Trichoderma* sp. ABC1 was reactivated in potato dextrose agar

Fig. 1. Collaborative participation among small producers in the community of Tixtla to install the demonstration plot.

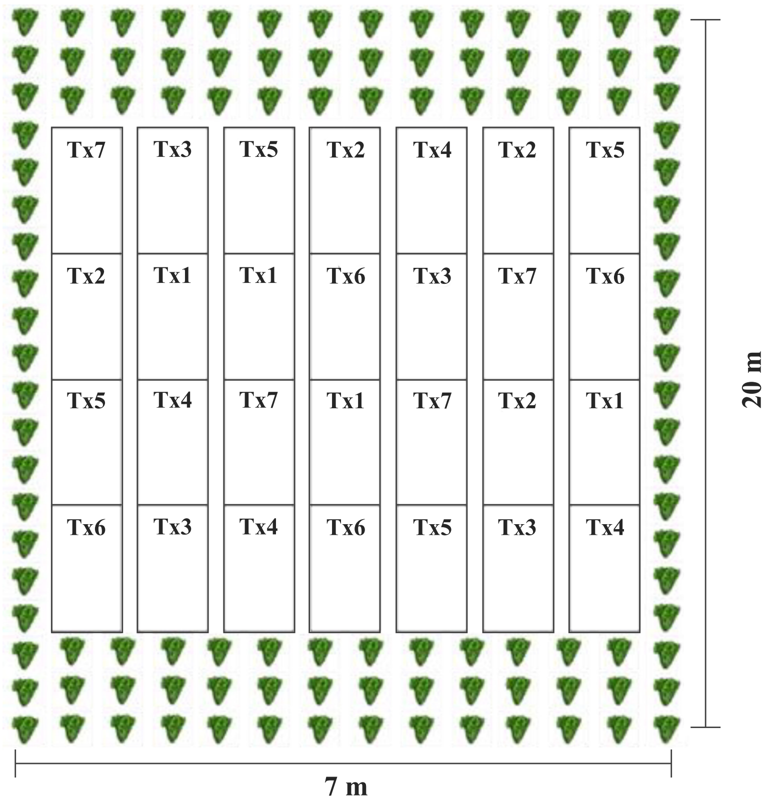


Fig. 2. Randomized Complete Block Design. Treatments were randomly distributed so they are repeated 4 times within the demonstration plot.

and incubated at 32 °C for 5 days (Pineda et al. 2017); its OD was estimated from 0.0 to 0.1 corresponding to 1×10^8 spores/mL (Michel et al. 2008).

To prepare the biofertilizers, the inoculate of the strains were placed in 100 mL of nutrient broth, 100 mL of 0.9% saline solution, 15 mL of molasses, and 5 g of salt in water for a final volume of 2 L and left to ferment for 5 to 7 days. The crops were maintained from germination to harvest following the agricultural practices of the region, which is why the germination of seeds with chemical fertilizer was not carried out, since the producers do not do it.

Seed germination test

The commercial seeds of the KristenSeed ® (for lettuce and celery) and Caloro ® (for epazote) brands were subjected to a viability test, and the percentage of germination higher or lower than 75% was determined as referenced by Salinas et al. (2001).

Seedling inoculation and biofertilizers application

The initial inoculation at the seed level was carried out by soaking them for 20 min in each treatment with NMPPG and in water for treatment six. In this case, the chemical treatment was not included since, according to the experience of the producer, no chemical treatment is applied to their seeds in the nursery as shown in **table I**. The initial inoculwwwation was carried out with fresh inoculate of 16 h adjusted to an OD from 0.08 to 0.1 (Martínez et al. 2020).

The second inoculation was carried out at the root level at the time of transplantation in a 25-day crop for lettuce, 20 days for epazote, and 40 days for celery. The roots were immersed in the biofertilizers for 20 min and a first application of 5 g per plant of the conventional fertilizer DAP-18-46-00 was applied to Tx6 in water. The third inoculation of 10 mL at the base of the stem was applied 25 days after the transplant and a second application of 5 g per plant of DAP-18-46-00 to Tx7.

Transplanting of seedlings, irrigation, and control of weeds

Lettuce was transplanted to the demonstration plot at 25 days, celery (40) and epazote (20) days respectively. Irrigation was done every third day,

TABLE I. FREQUENCY OF GERMINATION USING THE NMPPG IN LETTUCE, EPAZOTE AND CELERY.

Treatments	Germination rate (%)		
	Lettuce	Epazote	Celery
Tx1: *Azotobacter* sp. C3	80	73.7	70.6
Tx2: *Azospirillum* sp. M9	72.8	70.6	62.6
Tx3: *Rhizobium* sp. R01	93.7	82.2	80.4
Tx4: *Trichoderma* sp. ABC1	63.1	68.4	53.3
Tx5: *B. licheniformis* M2-7	70.6	79.1	69.3
Tx6: Water (negative control)	48.8	44	46.6
Tx7: DAP-18-46-00 (positive control)	ND	ND	ND

Notes: ND, not determined

and control of unwanted weeds was done manually every week.

Determination of germination index

Germination was done using the methodology described by Araya et al. (2000) using Eq. 1:

$$G = \frac{\textit{Germinated seeds}}{\textit{Seeds sown}} \times 100 \qquad (1)$$

Determination of fresh weight of each vegetable

The evaluation of fresh weight was carried out in the field at the time of harvest, removing excess soil and using a high precision HYIEAR digital scale, and the weight was recorded in g.

Estimation of vegetal yield

The yield was estimated considering the final weight obtained after harvesting each crop and the area used (Sanchez and Meza 2014). To calculate the yield in kg/ha and subsequently extrapolate it to ton/ha, we used Eq. 2.

$$\text{Yield}\left(\frac{kg}{na}\right) = \frac{\textit{Weight x plot (kg)}}{\textit{Plot area (m2)}} \times 10000\ \text{m}^2 \qquad (2)$$

Economic balance

To evaluate the costs generated in the crop and make an economic balance, the fixed and variable costs generated by the biofertilizers, and the purchase of fertilizers used by the producers were determined. The sale costs of the vegetables were established based on local market prices at the time of harvest (Hernández et al. 2018).

Statistical analysis

For the statistical analysis, analysis of variance (ANOVA) tests were performe, and for those variables that presented significant differences a test of multiple comparisons of means by Tukey's test with an alpha of 0.05. Statistical analysis was performed in GraphPad Prism 6.

RESULTS

We met with 50 producers to provide training on the use of biofertilizers based on NMPPG and an agreement was established with the president of the irrigation unit. The agreement consisted of the loan of an extension of agricultural land to establish the demonstration plot and the 3 crops of interest to the farmers. We worked directly with the farmers in the installation of the nurseries and demonstration plot (**Fig. 1**), and at the time of the harvest the germination index was determined as shown in **table I**, where we observed that Tx3, Tx1 and Tx5 were the best results in the three vegetables. Harvest and obtaining of the variables in epazote (2), lettuce (3), celery (6) months respectively.

At the end of the cycle of three months in lettuce, six months in celery and two months in epazote, each Tx was harvested, and the fresh weight was recorded. Tx1, Tx3 and 5 are the best in terms of fresh weight and foliage (**Fig. 3, 4, 5** and **6**).

The statistical analysis showed a value of $p < 0.05$ (**Table S1, S2** and **S3**) which indicates that there are differences between treatments. In general, all NMPPG treatments showed significant differences with respect to the negative control and the chemical control. Therefore, according to the statistical analysis, *Rhizobium* sp. R01 (Tx3), *Azotobacter* sp. C3 (Tx1) and *B. licheniformis* M2-7 (Tx5) were the best in the three vegetables (**Fig. 6**).

In the case of lettuce (**Fig. 6**), Treatment with *Azotobacter* sp. C3 showed differences with the rest of the treatments. Treatment with *Azospirillum* sp. M9 differs from treatment with *Rhizobium* sp. R01, *Trichoderma* sp. ABC1, *B. licheniformis* M2-7, Water and DAP 18-46-00. Treatment with *Rhizobium* sp R01 differs from treatment with *Trichoderma* sp. ABC1, *B. licheniformis* M2-7, Water and DAP-18-46-00. Treatment with *Trichoderma* sp. ABC1 differs from treatment with *B. licheniformis* M2-7, Water and DAP-18-46-00. Treatment with *B. licheniformis* M2-7 differs from treatment with Water and DAP-18-46-00 and treatment with Water differs from treatment with DAP-18-46 -00. This same pattern occurs in the cultivation of celery and epazote.

Yield was estimated considering the total harvested weight of all treatments in the demonstration plot for each vegetable, which was extrapolated to hectares in relation to the total value found in each experimental unit (Sánchez and Meza 2014). In all treatments, Tx3 stands out followed by Tx1 in the three vegetables with respect to all the others and the lowest yields were with water and fertilizer (**Table II**).

When the results of the yields were obtained in collaboration with the producers, the economic analysis was specified so that they could evaluate the economics of using the NMPPG as biofertilizer compared to the conventional fertilizer used.

The biofertilizer price was estimated considering the fixed and variable costs, which included: inputs, operating costs, packaging, labeling, and transportation, while the average cost of local distributors was used to set the cost of conventional fertilizer. The cost estimate was made considering a production area of 1.0 ha, finding that using both biofertilizers (5 liters) and conventional fertilizers (50 kg), the amount required costing $1,200.00 and $2,000.00 MXN respectively, however, other authors estimate that smaller presentations of biofertilizers (1000 mL) have an approximate cost of $200 MXN according to the estimate obtained by Orbe et al. (2022).

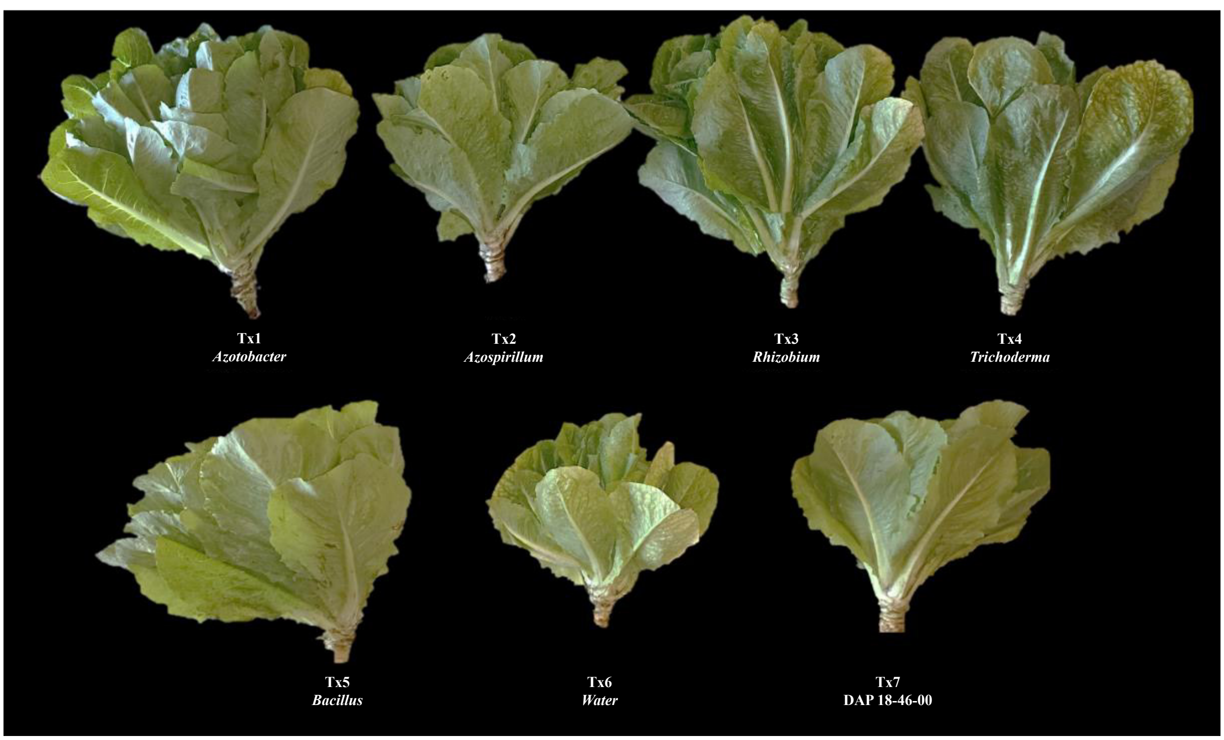


Fig. 3. Macroscopic morphology and foliage of lettuce harvested from each treatment.

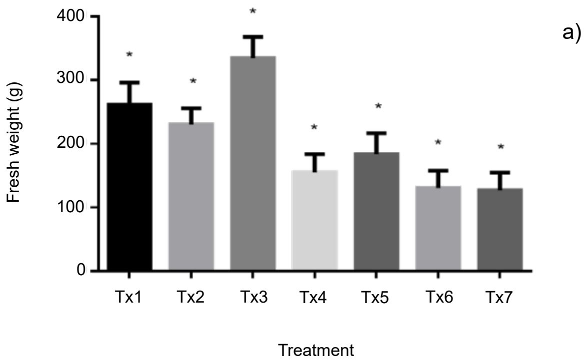
Fig. 4. Macroscopic morphology and foliage of epazote harvested from each treatment.

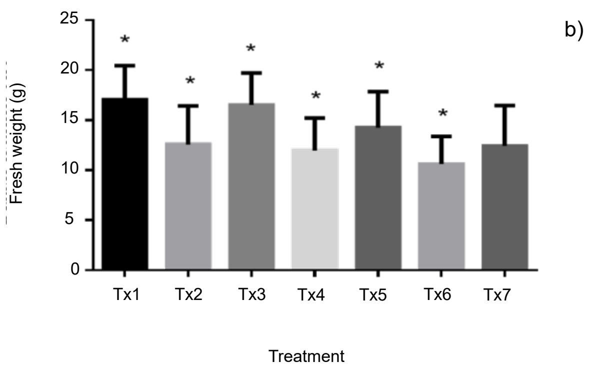
Fig. 5. Macroscopic morphology and foliage of celery harvested from each treatment.

DISCUSSION

The participation of the small producers of the irrigation unit during the project was fundamental for them to learn the application and use of NMPPG in vegetables, from germination to harvest, from the use of conventional fertilizer, water and biofertilizers and finally to see the results and the economic difference between the Tx, has been of great satisfaction, given that there are few studies where they are involved. Regarding the germination index considering the values of the percentage reported by Salinas et al. (2001) indices higher than 75% were obtained indicating that the seeds are of good quality and the Tx improve the index, in these results it is shown that Tx with *Rhizobium* sp. R01, and *Azotobacter* sp. C3 promoted better germination, coinciding with Burgos (2017) where he mentions that seeds of vegetable crops inoculated with these bacteria benefit from a higher germination percentage compared to uninoculated seeds.

The low germination rate of *Trichoderma* sp. ABC1 agrees with Gonzáles and Fuentes (2016) where they indicate that the treatments applied to rice, and cassava seeds were not effective to increase germination.





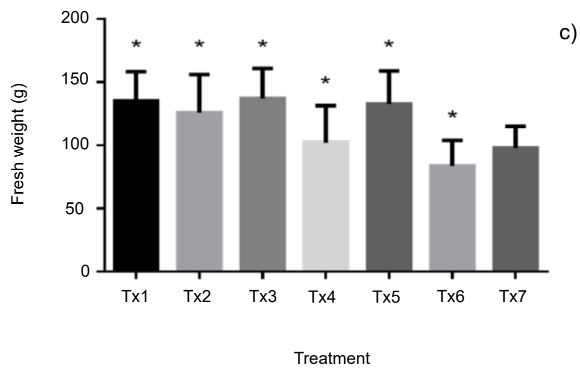


Fig. 6. Fresh weight (g) of: a) lettuce, b) epazote and c) celery plants. Treatments marked with * indicate significant differences.

TABLE II. EVALUATION OF YIELD FOR LETTUCE, EPAZOTE AND CELERY PER TREATMENT.

Treatments	Lettuce		Epazote		Celery	
	kg/ha	ton/ ha	kg/ha	ton/ ha	kg/ha	ton/ha
Tx1	1680.35	1.68	109.64	0.109	870.0	0.870
Tx2	1479.64	1.479	80.78	0.08	809.0	0.809
Tx3	2149.85	2.149	106.21	0.106	871.0	0.871
Tx4	996.85	0.996	76.92	0.076	656.0	0.656
Tx5	1182.21	1.182	91.64	0.091	853.0	0.853
Tx6	841.14	0.841	68.28	0.068	538.0	0.538
Tx7	817.35	0.817	79.92	0.079	629.0	0.629
TOTAL	9147.42	9.147	613.42	0.613	5266.0	5.226

NMPPG can establish in the rhizosphere and there is ample knowledge that they enhance plant growth and development through the direct and indirect functions they produce (Beneduzi et al. 2012). This promoting action is triggered when microorganisms produce and release phytohormones that help to capture nutrients and make them available for absorption by the roots, and indirect mechanisms when they help to reduce the impacts that some phytopathogens can generate; therefore, using these mechanisms, an NMPPG can act directly on the growth and development of crops by improving these two conditions (Glick 1995).

The production of plant hormones is an important characteristic in plant development since there is evidence that phytohormones such as gibberellins are involved in promoting germination (Cantaro et al. 2019., Saldivar et al. 2010), resulting in benefits for production. vegetable. Likewise, the activity of phytohormones such as auxins, gibberellins, promotion of Nitrogen fixation and phosphate solubilization produced by *Bacillus licheniformis*, *Pseudonomas putida*, *Pseudomonas* sp., *Enterobacter cloacae* and *Azotobacter vinelandii* is known in lettuce production in the state of Guerrero obtaining larger size and yield, concluding that these bacteria improve yield (Martínez et al. 2020).

Both *Rhizobium* sp. and Azotobacter sp. are microorganisms that have been used extensively in agricultural production, in the second case it is reported that they provide plants with up to 50% of nitrogen requirements and in both cases, they supply substances that stimulate plant development (Hayat et al. 2010). On the other hand, the action species of *Azospirillum* sp. and *Bacillus* sp. have also been reported because they are considered study models in their application as biofertilizers by direct mechanisms (production of gibberellins and auxins, nitrogen fixation and phosphate solubilization) and indirect (production of proteases, lipases, esterases, amylases, glucanases and chitinases) (Geetha et al. 2021). *Trichoderma* sp., have been used in management as biofertilizers, highlighting their capacity for biological control through indirect mechanisms (Argumedo et al. 2009).

Mexico is positioned as the ninth world producer of lettuce with a production of 542 tons for 2020, the average production in the producing states is 49.2 tons per hectare, the states of Guanajuato, Zacatecas and Aguascalientes are the largest national producers with 149.7, 96.1 and 79.9 tons per hectare respectively; of this production only 8.56 tons/ha of irrigation and 13.9 tons/ha of rainfed were planted in Guerrero (SIAP 2020). Our results show an increase in lettuce biomass with the application of NMPPG as observed in **Table II**, increasing the yield with rainfed irrigation, which would open a perspective to estimate if the application of our biofertilizer would increase lettuce production in rainfed irrigation since it is one of the most cultivated crops in the study area and because it is considered of commercial interest, it was selected to be evaluated, obtaining that the yield increases when working with these microorganisms.

The main producing states of celery are Guanajuato, Baja California, Sonora, Mexico City, Jalisco, Michoacán, Queretaro, Zacatecas, and Puebla, considering its production in 2020, yields of 42.4 tons per hectare were obtained (SIAP 2020) a higher figure to the one obtained in our research, where it should be noted that the main cultivated variety is *Apium graveolen* var. sweet characteristic due to the fleshy and large volume stems (INTAGRI 2021) that increase the fresh weight compared to the secalinum variety, which was used for this research whose importance at the morphological and nutritional level lies in the leaves and develops in a smaller size. (Martínez et al. 2016) which could be an important factor when comparing the yield of both crops. As the state of Guerrero is not included in the list of celery producers at the national level, these results can be integrated into the production system of the Juan Berbera Catalan Irrigation unit since celery has not been considered as a crop of interest.

In the case of epazote, in 2020 yields of 11.04 tons per hectare were obtained and where the main producing and exporting states are Puebla, Tlaxcala and the State of Mexico (SIAP 2020) among which the state of Guerrero does not appear as a producer.

In this investigation we obtained a total of 0.613 ton/ha, a figure lower than that estimated at the national level; however, according to the information collected from the producers, that due to the atypical rains that occurred in this area during some months of 2022, the few epazote crops, despite being short harvests, were affected, decreasing the yield, so the use of NMPPG is recommended, it may represent an option to implement in the strategy to increase the yield of this crop.

During 2021 in the municipality of Tixtla, both irrigated and rainfed farmers sowed 4,888.99 hectares of land and harvested the same amount of variety of crops including flowers, vegetables, and grains (García 2022). However, an estimate of the total yield of these crops has yet to be made, so it would be important to consider alternatives to increase fresh weight for better yields using biofertilizers with NMPPG in the subsequent sowing of various crops in this area.

Considering the distribution system of the vegetables in a randomized complete block design with measures of 7 × 20 m, each block has measures of 5 × 1 m distributed in 7 furrows with 4 blocks respectively. Extrapolating this data to hectares and tons in 10 000 m² could be distributed up to 50 000 plants which would increase the yield and in turn the economic income.

The use of chemical fertilizers has increased due to the high demand for agricultural products which must add a variety of quantities of this input to obtain high yields with greater crop efficiency, causing agriculture to become highly dependent on these fertilizers (Kang et al. 2021). Therefore, it is currently required to promote more economical uses without neglecting the efficacy and reliability of the products applied to crops and that optimize root development, strengthen the response against diseases, pests, increase yields and reduce costs (Bagyalakshmi et al. 2017).

The price of fertilizers has increased drastically, by 89% in 2021, so, the higher the price of fertilizers, the higher the production costs for the pockets of producers and, therefore, higher prices to the products which represents a problem in the agricultural sector due to the lack of economic incentives, government support and economic losses.

The use of NMPPG as an alternative of constant use in the application of the studied crops and others can represent a reliable alternative due to its low cost and time of use in addition, the economy of the producers will be favored with an estimated savings of $800 MXN for an application per hectare in these three vegetables used, and/or a combination of both, since DAP 18-46-00 (diammonium phosphate with nitrogen (18%) and phosphorus (46%) provides nutrients to the soil, and favors the increase of NMPPG in agricultural soils to improve the availability and use of nutrients by plants. It is important to mention that for each liter of biofertilizer it can be increased up to 10 liters in optimal conditions for the development of microorganisms, which is why it represents a viable option to be implemented in crops. Other authors estimate that smaller presentations of biofertilizers (1000 mL) have an approximate cost of $200 MXN according to the estimate obtained by Orbe et al. (2022), generating a difference positive of $8,450.00 MXN using biofertilizers.

Regarding the profits of each crop, celery and epazote are not harvested by many producers, but they are interested in proving their economic benefits, and finally the costs of lettuce depend on local competition and demand and can reach a cost up to $10.00 MXN per piece and in seasons with lower demand it can be sold between $50.00 and $20.00 MXN for every dozen lettuce depending on the size. In addition to the decrease in prices, the application of the NMPPG represents an importance in reducing adverse effects on the environment since its excessive application has produced a variety of environmental problems such as contamination of water bodies, air pollution, soil degradation and negative effects impacts on ecosystems, ecological imbalance, reduction of biodiversity and health problems for people who are exposed to the constant application of fertilizers (García and Rodríguez 2012).

CONCLUSION

The use of biofertilizers made with NMPPG represents an effective option to be implemented in agricultural production by small producers in the community of Tixtla in Guerrero, since it improved germination, fresh weight, foliage and yields; furthermore, NMPPG biofertilizers are easy to apply, cheaper, and environmentally friendly. Moreover, the collaboration with the producers in the demonstration plots directly allowed the farmers to transition from conventional fertilizers to the use of NMPPG to improve quality and reduce crop costs.

ACKNOWLEDGMENTS

We thank the members of the Juan Berbera Catalán Irrigation Unit and Conahcyt for financing this project through its scholarship program.

We especially thank PhD. Diana Iveth Orbe Díaz, PhD, Victor Manuel Rosas Guerrero, and MSc. Jazmin Alaide López Díaz for her advice on this research. To the work team that makes up the Laboratory of Molecular Microbiology and Environmental Biotechnology Msc. Amayrani López Carrasco, PhD. Yadciri Ojendiz Mata, BS. Andrea Loaeza Nájera and MSc. José Alberto Villanueva Solano for their support and company in the field work.

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SUPPLEMENTARY MATERIAL

Table S1. ANOVA ANALYSIS FOR LETTUCE CROP.

LETTUCE					
ANOVA	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	3154000	6	525622	F (1.376, 122.4) = 1822	P < 0.0001
Individual (between rows)	415500	89	4669	F (89, 534) = 16.18	P < 0.0001
Residual (random)	154071	534	288.5		
Total	3723000	629			

Table S2. ANOVA ANALYSIS FOR CELERY CROP.

CELERY					
ANOVA	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	247907	6	41318	F (6, 622) = 67.96	P < 0.0001
Residual (within columns)	378146	622	608		
Total	626053	628			

Table S3. ANOVA ANALYSIS FOR EPAZOTE CROP.

EPAZOTE					
ANOVA	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	3138	6	523	F (2.819, 250.9) = 731.1	P < 0.0001
Individual (between rows)	7131	89	80.12	F (89, 534) = 112.0	P < 0.0001
Residual (random)	381	534	0.7153		
Total	10651	629			