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Evaluation of the efficiency of rhizobial biofertilizers in guava crop (*Psidium guajava* L.) using statistical quality control

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ABSTRACT

Objective: Apply statistical quality control to evaluate the efficiency of biofertilizers for the sustainable development of crops of guava (*Psidium guajava*) in Chiapas, Mexico.

Design/methodology/approach: Physicochemical parameters were analyzed to determine soil fertility in guava crops. The structure and diversity of the bacterial community was studied by structural metagenomics. A quality control statistical analysis was applied to determine the effect of biofertilization on the growth and production of the guava plant crop.

Results: The soils were silty clay and had variations in pH and cation exchange capacity. Guava plants inoculated with PGPB rhizobial bacteria had higher growth and number of fruits. The cause-effect analysis determined that soil nutrients, plot phytotechnical management and bacterial diversity significantly influence the effectiveness of biofertilizers.

Limitations on study/implications: Atypical climatic variations in the region, deficient pest control and high genetic variability in plants influence the productivity of guava crops. It is important to explore a larger area of crops to detect more cause-effect elements.

Findings/conclusions: Experimental statistical analyzes and quality control are effective tools to determine the efficiency of biofertilizers in fruit crops.

Keywords: Guava crop, biofertilizers, quality control, rhizobial bacteria.

INTRODUCTION

Conventional agricultural systems in Mexico demand the use of large amounts of fertilizers and a wide variety of agricultural inputs in order to maintain crop yields (Arriaga *et al.*, 2017). The constant and systematic use of these agricultural inputs causes alterations in the soil fertility and unfortunately severe damage to human health and

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the environment (Mozumder and Berrens, 2007). Biofertilizers emerge as a sustainable biotechnological alternative to reduce the use of fertilizers and improve the yield of crops (Itelima *et al.*, 2018). Biofertilizers are beneficial microorganisms that promote plant growth and improve soil health. Bacteria known as plant growth promoters (PGPB) are commonly used as biofertilizers (Vessey, 2003). Among the PGP bacteria, the *Rhizobium* genus stands out for its ability to fix nitrogen, solubilize phosphate, among other biological qualities (Chen *et al.*, 2003). Several authors have reported the positive effect of biofertilization on different types of agricultural crops. However, information on the application of biofertilizers formulated with *Rhizobium* bacteria in fruit crops is very scarce (Rincón-Molina *et al.*, 2022). In southern Mexico, fruit growing is one of the most important agricultural activities since it strengthens food security and the economy at a regional and national level. The cultivation of the guava in the metropolitan region of Chiapas, Mexico, has increased in recent years, becoming a strategic crop with high agricultural and socioeconomic potential (Rincón-Molina *et al.*, 2022). Guava (*Psidium guajava* L.) belongs to the family Myrtaceae, which includes more than 70 genera and approximately 2,800 species (Vitti *et al.*, 2019). Guava fruit is rich in antioxidant activity, maybe due to its high vitamin C content. This crop requires important conditions to achieve good yields, mainly that the soils have a good nitrogen and potassium content (Montes *et al.*, 2016). Local guava farmers apply large amounts of chemical fertilizers to increase crop yields. However, despite this agricultural practice, the desired results have not been achieved and, on the contrary, the deterioration of the soil and the contamination of the environment have increased. The application of biofertilizers in guava crops emerges as an important agrobiotechnology for the improvement and sustainable development of this type of fruit crops (Mosa *et al.*, 2021). In agricultural production systems, the modern use of quality control tools makes it possible to identify, correct and improve those factors that negatively influence crop yields (Miroslav *et al.*, 2016). In this way, it is possible to apply the continuous improvement of the processes. In this work, statistical designs and cause-effect quality control (Ishikawa diagram) were applied to evaluate the efficiency of rhizobial biofertilizers applied in a guava crop

MATERIALES Y MÉTODOS

Experimental site

The biofertilization trials were carried out in an agricultural field of guava cultivation called “Rio Grande” located in the Ribera de Monte Rico in Chiapas, Mexico (16° 71’ 04” N and 93° 03’ 12” W), at an average height of 400 m.a.s.l (Figure 1). The inoculation tests and the different samplings were carried out in an area of 2100 m^2.

Soil characterization

Rhizospheric soil samples were collected from five randomly located points in the guava experimental plot 20 cm deep from the top layer. The samples collected from each point were mixed to obtain a single representative sample that was used for a chemical fertility analysis. The pH and electric conductivity (EC) were measured by using a digital pH meter. Cation exchange capacity (CEC) was determined according to the Official Mexican

Figure 1. Location of the experimental plot of guava cultivation.

Standard NOM-021-SEMARNAT-2000. The determination of total nitrogen and total carbon was carried out by using a FLASH 2000® auto-analyzer. Total phosphorus was determined with the solubilization method of $HNO_3/HClO_4$ (Rincón *et al.*, 2020). Additionally, soil samples were obtained to study the structure and diversity of the bacterial community through a metagenomic analysis (Rincón-Molina *et al.*, 2022).

Bacterial strain used in biofertilization trials

The bacterial strain *Sinorhizobium mexicanum* ITTG-R7 (DQ411930) was used in biofertilization trials. The strain was isolated from the legume *Acaciella angustissima* and is characterized by its high nitrogen fixation and plant growth promoting capacity (Rincón-Rosales *et al.*, 2021).

Experimental design for guava crop biofertilization

Inoculation trials were conducted at the experimental plot on 4-year-old guava plants, which were uniform in size and vigor, and spaced 7.0 m between the rows and 7.0 m between plants. The experimental unit consisted of one guava tree. A completely randomized design was used for the experiment with six replications of each treatment. In this experiment, the effects of five treatments on the growth of guava plants were evaluated. Treatments consisted of: T_1: (*S. mexicanum* ITTG-R7), T_2: (ITTG-R7 + Fertilizer Triple 17), T_3: (*Azospirillum brasilense* CD), T_4: (Triple 17), and T_5: [non-inoculated and non-chemically fertilized plants]. The strain *A. brasilense* CD which is commercially available, was applied as a PGPB reference. The guava plants were inoculated with *S. mexicanum* ITTG-R7 and *A. brasilense* CD strains to a concentration of 10^6 CFU mL^{-1}. Plants were inoculated with 100 mL of bacterial suspension, which was applied directly to the plant base. Every 3 months, the plants were inoculated over an experimental period of 9 months. In the same way, triple 17 fertilizers, was applied to the plants using a fertigation system. At the end of the experiment, the variables: total plant height, foliar cover, basal diameter, flowers number, fruits number and total chlorophyll were determined. Data obtained from the inoculation test were analyzed by ANOVA at a significance level of alpha=0.05 by using the statistical software Statgraphics Centurion XV.2. The comparison of means was carried out by the Tukey test ($p<0.05$).

Quality control analysis applied to the biofertilization process

Statistical analysis was applied to determine the Quality Control Limits (QCL) to the morphometric variables (plant height, foliar cover, basal diameter) and number of fruits in the guava cultivation system to know if the production is adequate or requires some improvement (Gutiérrez and De La Vara, 2013). Also, a cause-effect analysis (Ishikawa diagrams) was applied to detect the causes that affect crop yield in the guava production system and thus establish countermeasures to improve the efficiency of biofertilization (Acosta *et al.*, 2019).

RESULTADOS Y DISCUSIÓN

The soil sample obtained from the guava plot was silty clay loam in texture, shallow and with good water drainage. The pH was slightly acidic [6.1±(0.022)] and the EC was 0.82±(0.021) dSm^{-1}. Guava grows well in a wide range of soil types, but prefers well-draining soil with a pH between 5 and 7 (Shukla *et al.*, 2014). The cation exchange capacity (CEC) value was 26.32±(0.011) Cmol kg^{-1}. In relation to the fertility parameters N, C, and P. The chemical analysis indicated low levels for the content of total N [0.18%±(0.017)] and for C content [0.91%±(0.012)]. In the case of available P, high value [52.06 mg kg^{-1}±(0.328)] was determined. The C:N ratio is considered as an important parameter related to soil fertility. In this study, the soil of the guava crop had a low value of the C:N ratio (5.0±0.18) according to the Official Mexican Standard NOM-021-SEMARNAT-2000, indicating a rapid mineralization and release of N, which is available for plant uptake.

Regarding the study of the bacterial community of the rhizosphere soil of the guava crop. 16S rRNA gene sequences showed a wide diversity of bacterial species in the soil of the guava crop. Actinobacteria was the most abundant phylum (>20% relative abundance) followed by Proteobacteria, Acidobacteria, and Firmicutes (Figure 2).

Bacillaceae family dominated in soil samples. Gaiellaceae and Vicinamibacteraceae, which were also found, are related to carbon transformation in soil. Among the identified bacterial genera, *Bacillus* is characterized by its high potential as PGPB. These bacteria have the ability to produce auxins and other plant growth promoters (Rincón-Molina *et al.*, 2022).

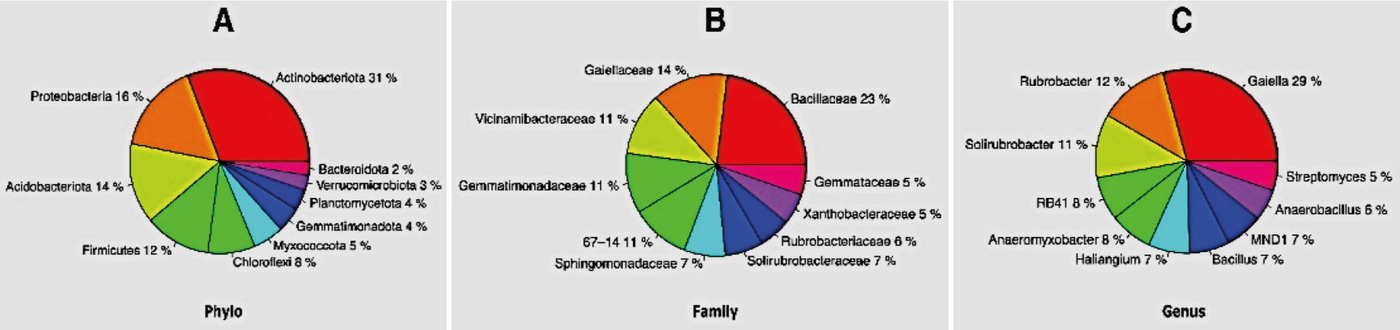

Figure 2. The bacterial community structure of the rhizospheric soil of the guava crop.

Biofertilization with diazotrophic bacteria in guava cultivation positively influenced the growth of guava plants (Table 1).

The plants inoculated with ITTG-R7 strain had a higher height compared to the other treatments. The application of the ITTG-R7 strain alone or mixed with the fertilizer (Triple 17) had a significant effect ($p<0.05$) on the foliar cover of plants. The basal diameter of the stem increased in those plants that were treated with both the fertilizer as well as with the biofertilizer. A significant effect ($p<0.05$) was observed in the plants treated with the ITTG-R7 strain in relation to the number of flowers. Trees inoculated with *S. mexicanum* ITTG-R7 strain as well as those chemically fertilized registered a significant increase in the number of fruits compared to the control plants (without fertilization/uninoculated). The amount of total chlorophyll was higher in the plants inoculated with *S. mexicanum*. The positive effect on growth in guava was observed with the inoculation of ITTG-R7 mixed with chemical nutrients. Similar effects have been found in fruit crops inoculated with *A. brasilense*. For fruit crops, it has previously been reported that combinations of biofertilizers with nitrogen and other nutrients provide the best effects on plant development, and thus, on yields (Dwivedi *et al.*, 2012). Bacteria as biofertilizers with plant growth potential when combined with inorganic and organic inputs help with crop nutrient uptake; thus the results suggest that ITTG-R7 plays a key role in nutritional improvement.

In relation to the application of quality control analysis in an agricultural production system. In the first instance, we determined the quality control limits for different growth and production parameters in guava crop plants that were biofertilized with the *S. mexicanum* ITTG R7 (Figure 3).

For the total plant height, it was determined that 82% of the plants were within the central limit (CL). For leaf cover, the CL value was 85%. Basal diameter was above 84%. For the number of fruits, a CL value of 86% was obtained and for the chlorophyll

Table 1. Effect of biofertilization on growth parameters in guava plants.

Treatment	Plant height (cm)	Foliar cover (cm)	Basal diameter	Flowers number	Fruits number	Total Chlorophyll (mg mL^{-1})
T1 (Strain ITTG R7)	369 a*	553 a	98 ab	36 a	63 a	2.8 a
T2 (ITTG R7 + Triple 17)	327 b	516 ab	107 a	29 b	53 ab	3.7 b
T3 (*A. brasilense* + Triple 17)	312 b	462 b	64 c	22 c	35 bc	2.6 b
T4 (Fertilizer Triple 17)	336 b	475 b	86 b	23 bc	50 ab	2.7 b
T5 (Negative control)	267 c	375 c	57 c	19 c	25 c	2.0 c
p-value	0.000	0.005	0.004	0.003	0.001	0.001
HSD ($p\leq0.05$)	24.7	58.5	12.1	6.73	20.0	0.31

*Mean values of six replicates. Means followed by the same letter are non-significant (HSD Tukey test, $p\leq0.05$).

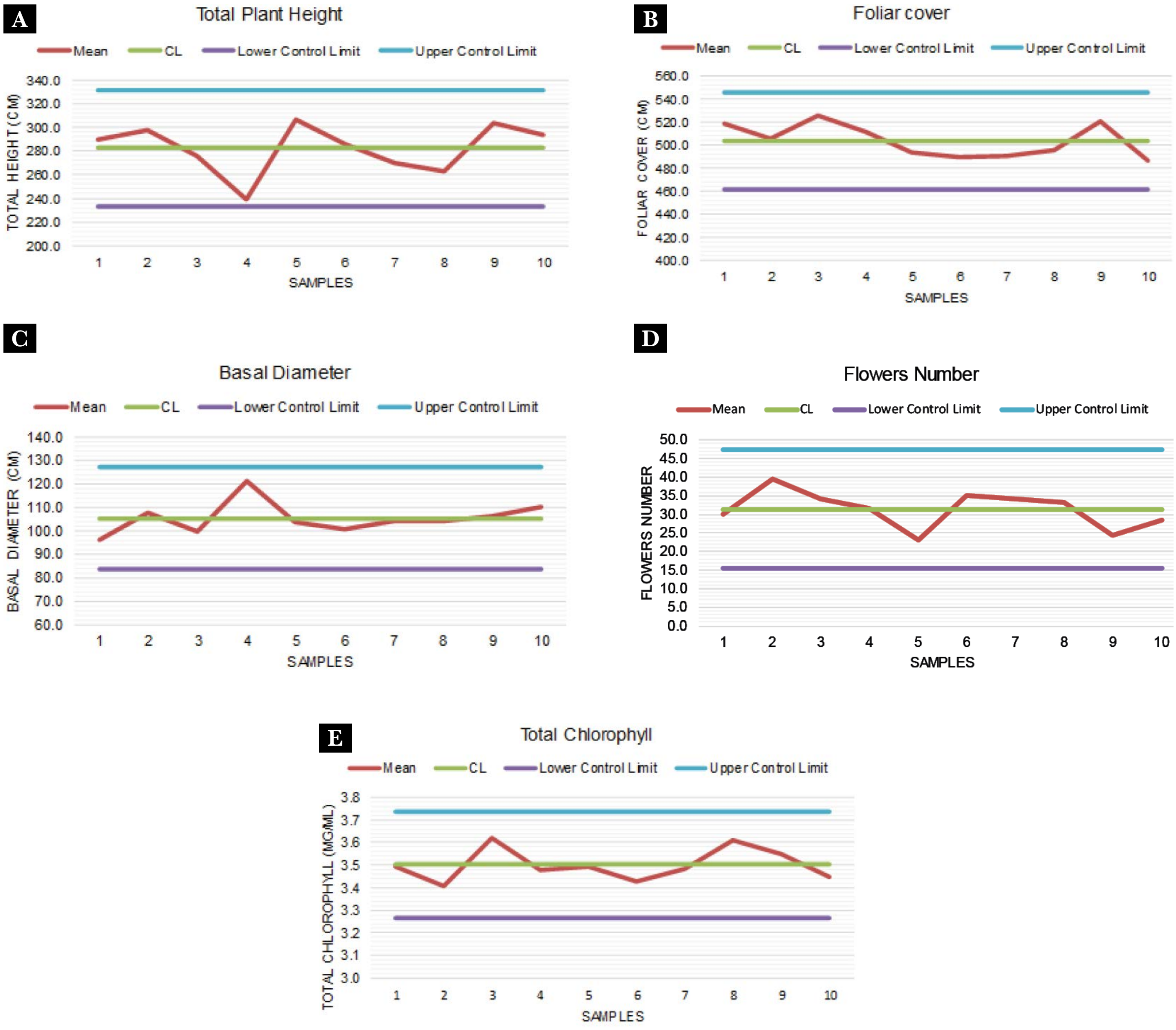

Figure 3. Quality control charts for development and production parameters in a biofertilized guava crop.

content the CL value was 84% (Figure 3). In general, in statistical quality control analyzes it is important to maintain a confidence level (1-alpha) greater than 95%, in this case we consider that biofertilized guava plants showed a positive effect on growth and production. However, it is necessary to implement some additional measures to improve the production process.

On the other hand, cause-effect analysis allowed us to determine that phytotechnical management, agricultural inputs, soil nature, labor, raw material were the main causes that are related to yield in guava cultivation. In Figure 4, an Ishikawa diagram is shown, with the effects main causes and the sublevels in each of these. In this work it was possible to apply countermeasures especially for the phytotechnical management of plantations and programs to improve crop growth and yield through the detection and elimination of pests, weeds and control of birds and insects that damage plants and fruits. Likewise, it was observed that the application of biofertilizers positively influence plant growth, flowering, and fruit production.

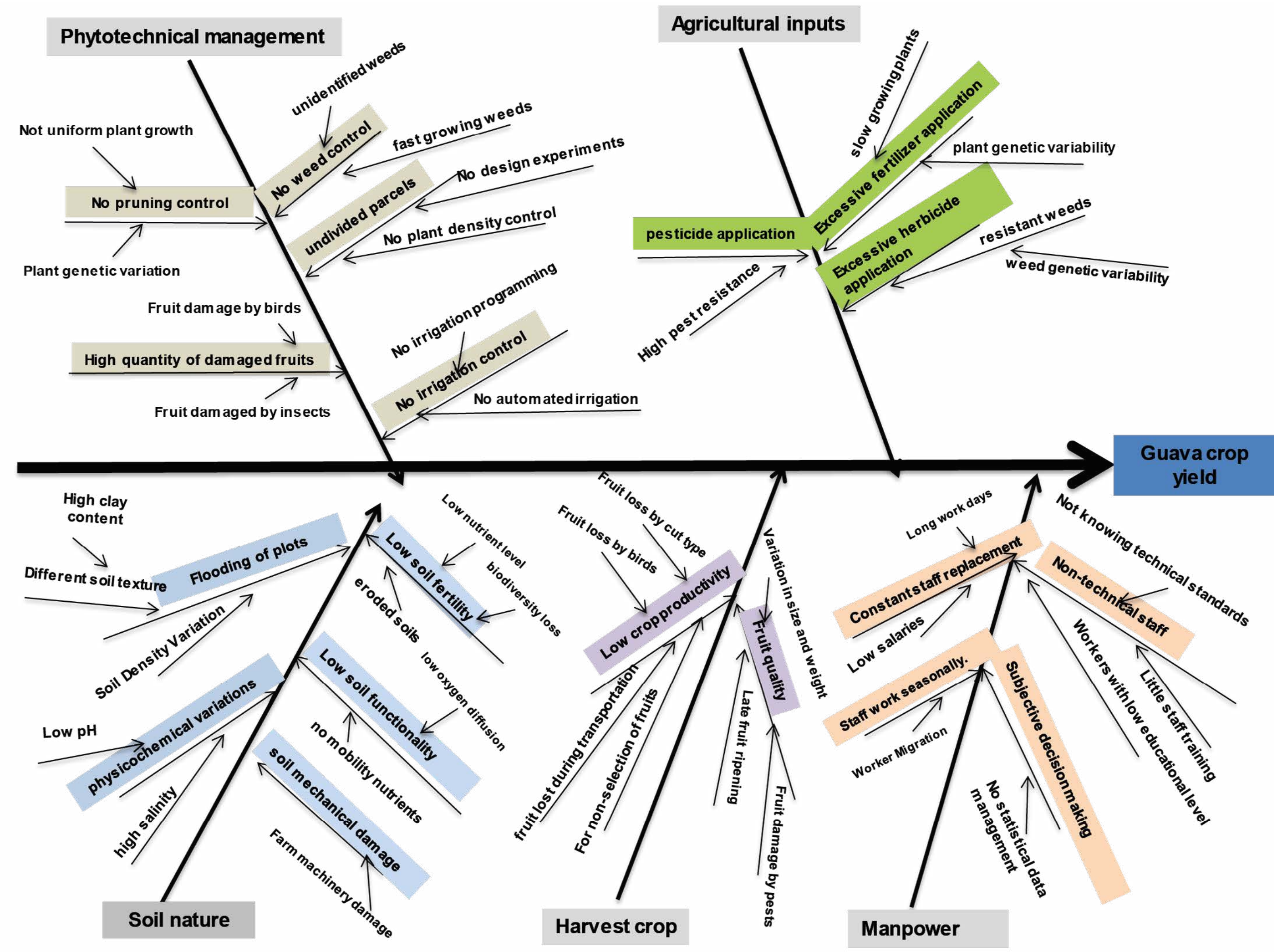

Figure 4. Cause-effect diagram (Ishikawa) on guava crop production process.

CONCLUSIONS

Rhizobial biofertilization significantly improved the growth and production of the guava crop. *S. mexicanum* ITTG-R7 stood out as a PGPB species that can contribute to the growth of guava plants and also improve the quality and functionality of the soil. Phyto technical management, agricultural inputs, soil nature, labor, raw material were the main causes that are related to low yield in guava cultivation. Experimental statistical analyzes and quality control are effective tools to determine the efficiency of biofertilizers in fruit crops.

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REFERENCES

Acosta, Q.M., Emmerth, O.D., Naranjo, F.A., Lizardi, D. M. (2019). Mejoras para la reducción de defectos de calidad de una empacadora de hortalizas. *Theorema*, 186-193.

Arriaga, F.J., Guzman, J., Lowery, Birl. (2017). Conventional agricultural production systems and soil functions. Chapter 5. Soil Health and Intensification of Agroecosytems, *Academic Press*, P:109-125, doi.org/10.1016/B978-0-12-805317-1.00005-1.

Chen, W.M., Moulin, L., Bontemps, C., Vandamme, P., Béna, G., Boivin-Masson, C. (2003). Legume symbiotic nitrogen fixation by β-proteobacteria is widespread in nature. *J. Bacteriol.* 185, 7266–7272. doi.org/10.1128/JB.185.24.7266-7272.2003.

Dwivedi, D.H., Lata, R., Ram, R.B., Babu, M. (2012). Effect of biofertilizer and organic manures on yield and quality of Red Fleshed Guava. *Acta Hort*. 933, 239–244.

Gutiérrez Pulido, H., & De la Vara Salazar, R. (2013). Control Estadístico de la Calidad y seis sigma. México: Mc. Graw Hill. 325 pp.

Itelima, J., Bang, W.J., Onyimba, I.A., Sila, M.D., Egbere, O.J. (2018). Bio-fertilizers as key player in enhancing soil fertility and crop productivity: A review. *J. Microbiol. Biotechnol. Rep.* 2, 22–28.

Miroslav, P., Kotorová, Martina., Savov, Radovan. (2016). Quality Control in Production Processes. *Acta Technologica Agriculturae*. 3: 77–83. doi 10.1515/ata-2016-0016.

Montes, R.M., Parent, L.E., Amorim, D.A., Rozane, D.E., Parent, S.E., Natale, W., Modesto, V.C. (2016). Nitrogen and potassium fertilization in a Guava orchard evaluated for five cycles: Effects on the plant and on production. *Rev Bras Cienc Solo*. doi: 10.1590/18069657rbcs20140532.

Mosa, W.F.A., Sas-Paszt, L., Górnik, K., Ali, H.M., Salem, M.Z.M. (2021). Vegetative growth, yield, and fruit quality of guava (*Psidium guajava* L.) cv. Maamoura as affected by some biostimulants," *BioResources 16*(4), 7379-7399.

Mozumder, P., & Berrens, R.P. (2007). Inorganic fertilizer use and biodiversity risk: An empirical investigation. *Ecol. Econ*. 62, 538–543. doi.org/10.1016/j.ecolecon.2006.07.016.

Rincón-Molina, C.I., Martínez Romero, E., Ruiz-Valdiviezo, V.M., Rogel-Hernández, M.A., Villalobos-Maldonado, J.J., Rincón-Rosales, R. (2020). Plant growth-promoting potential of bacteria associated to pioneer plants from an active volcanic site of Chiapas (Mexico). *Appl. Soil Ecol.* 146, 103390. doi.org/10.1016/j.apsoil.2019.103390.

Rincón-Rosales, R., Rogel, M.A., Guerrero, G.; Rincón-Molina, C.I., Lopez-Lopez, A., Manzano-Gómez, L.A., Ruiz-Valdiviezo, V.M., Martinez-Romero, E. (2021). Genomic Data of *Acaciella* Nodule *Ensifer mexicanus* ITTG R7T. *Microbiol. Resour. Announc.* 10, e01251-20. doi.org/10.1128/MRA.01251-20.

Rincón-Molina, C.I., Martínez-Romero, E., Manzano-Gómez, L.A., Rincón-Rosales, R. (2022). Growth Promotion of Guava "Pear" (*Psidium guajava* cv.) by *Sinorhizobium mexicanum* in Southern Mexican Agricultural Fields. *Sustainability*, 14, 12391. doi.org/10.3390/su14191239.

Shukla, S.K., Adak, T., Singha, A., Kumar, K., Singh, V.K., Singh, A. (2014). Response of guava trees (*Psidium guajava*) to soil applications of mineral and organic fertilisers and biofertilisers under conditions of low fertile soil. *J. Agric. Res.* 22, 105–114. doi.org/10.2478/johr-2014-0027.

Vessey, J.K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil.* 255, 571–586. doi.org/10.1023/A:1026037216893.

Vitti K. A., Maluf de Lima. L , João Gomes Martines, J.F. (2019). Agricultural and economic characterization of guava production in Brazil. *Revista Brasileira de Fruticultura. 42*(1). doi:10.1590/0100-29452020447.