Open Access Library Journal
2022, Volume 9, e9069
ISSN Online: 2333-9721
ISSN Print: 2333-9705

Bio-Fertilization Effect on the Foliar Content of Nitrogen (N), Phosphorus (P) and Potassium (K) of Two QPM Maize Varieties in Two *Luvisols* of Yucatan, Mexico

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How to cite this paper: Ramírez-Silva, J.H., Ramírez-Jaramillo, G. and Lozano-Contreras, M.G. (2022) Bio-Fertilization Effect on the Foliar Content of Nitrogen (N), Phosphorus (P) and Potassium (K) of Two QPM Maize Varieties in Two *Luvisols* of Yucatan, Mexico. *Open Access Library Journal*, **9**: e9069.
https://doi.org/10.4236/oalib.1109069

Received: July 1, 2022
Accepted: August 8, 2022
Published: August 11, 2022

Abstract

The efficiency of two Quality Protein Maize (QPM): Sac Beh (*Sac*) and Chichen Itza (*Chich*) to extract nutrients from the soil and export to the plants was evaluated by applying Bio-fertilizers (*Bio*) in combination with Chemical fertilizers (*Chem*) in two *rhodic Luvisols* of Yucatan Mexico with low (Lot 1) and high (Lot 2) intensive agriculture use. This work was conducted in the Uxmal Experimental Station of Yucatan Mexico. Three treatments were evaluated: 1) the Control, No *Chem* no *Bio*, 2) *Chem* (60-80-00) of Nitrogen (N), Phosphorus (P_2O_5) and Potassium (K_2O), and 3) the combination of *Bio* plus *Chem* (60-80-00 + mycorrhizal fungi + azospirillum bacteria) distributed in a Randomized Block Design with three repetitions. At silk stage, the opposite leaves of the ears were sampled and analyzed for Nitrogen (N), Phosphorus (P) and Potassium (K) reported in percentage (%) and compared with Critical Levels. The yields ($t \cdot ha^{-1}$) were matched with the nutrient contents. The *Sac* was more efficient to extract N from the soil and exported to leaves than *Chich* in Lot 1 but *Chich* was more efficient than *Sac* in Lot 2. The two varieties showed foliar N contents below the critical levels in both lots, even with the application of fertilizers. In Lot 2 with higher P in the soil, any plant showed deficiencies including the Control (00-00-00). Deficiencies of K were determined in *Sac*-Lot 1 (1.60%) and *Chich*-Lot 2 (1.56%) just in the control (00-00-00) but not in *Chem* and *Chem-Bio*. This suggests that the absorption of native K in the soil was encouraged by the application of *Chem* and *Bio*. The deficiencies of K in the Control can be attributed to an antagonistic effect of the high contents of Calcium (Ca) and Magesium (Mg) over K in the soil.

Subject Areas

Agricultural Engineering

Keywords

Nutrimental Efficiency, Nutrient Availability, Mycorrhizae, Azospirillum Bacteria

1. Introduction

In Mexico, approximately more than 7 million hectares are annually planted with corn [1] in a wide diversity of environments that range from sea level to the highest valleys with more than 2200 meters above sea level and great variation in climate and rainfall [2].

In the Yucatan Peninsula of Mexico, more than 354,000 hectares of corn are annually sown [1]; however, native varieties of conventional grain with low yield potential and poor protein quality continue to be cultivated on a larger area.

This happens even when interdisciplinary research has been carried out through breeding programs where conventional native corn has been converted to varieties with higher protein quality (Lysine and Tryptophan) called: "High Quality Protein Corn" (Quality Protein Maize, QPM) [3]. The QPM corn was developed, for specific regions, where corn is the staple food of humans, although various studies have indicated its positive impact on weight gain of poultry and pigs [4].

In recent years, the National Institute of Forestry, Agricultural and Livestock Research (INIFAP) has developed, for different regions of Mexico, varieties of high genetic, physiological and sanitary quality with higher yields and better economic profitability [3]. Producing these materials has the purpose to benefit the social and economic marginal areas of Southeast Mexico to cope with the problem of human malnourishing [3].

Sac Beh (white corn) and *Chichén Itzá* (yellow corn) varieties were developed by genetically introducing 75% of a Mayan Creole germplasm and 25% of a high-quality protein donor named Hibrid-519 C. They have innate characteristics of native corn adapted to the stony areas where peasants are practicing shifting cultivation (slash and burn) as an alternative to improve their standard of family living [5].

For corn to be considered as QPM one, it must have levels of lysine and tryptophan greater than 0.35 and 0.072 g 100 g^{-1} [6]. INIFAP varieties can produce 2.5 $t \cdot ha^{-1}$ on rocky soils and a little more than 5.0 $t \cdot ha^{-1}$ on deep soils such as *rhodic Luvisols* [5].

With those varieties exist the potentiality to use Bio-fertilizers alone or combined with chemical fertilizers in order to enhance yields; however, specific studies are required from a nutritional and scientific point of view.

The nutrients that most limit the productivity of crops in various regions of

Mexico are nitrogen and phosphorus [7] which are used excessively by producers (chemical fertilizers), with a negative impact on the environment and low profitability due to a continuous rise of chemical fertilizers costs.

Faced with these problems, the understanding of the nutritional dynamics occurring into the plants is highly needed when fertilizers programs are about to be launched. This work aimed to evaluate the effect of Bio-fertilizers in the nutritional content of nitrogen, phosphorus and potassium in two QPM corn varieties when planted on two *rhodic Luvisols* in Yucatan, Mexico.

2. Materials and Methods

2.1. Location and Agroecosystems Studied

The study was carried out in the south region of the state of Yucatan, Mexico at the Uxmal Experimental Station of INIFAP located in the municipality of Muna (20˚29'08.1" north latitude and 89˚24'39" west longitude) at an altitude of 50 meters above sea level. The experimental plots were established on red soils (**Figure 1**) classified as *rhodic Luvisols* differentiated by their low (Lot 1) and high (Lot 2) intensive agricultural use.

Both lots have important chemical differences as shown in **Table 1**. Even though, both lots have neutral pH, the salinity of Lot 1 is low according to the Electrical Conductivity (EC) of 0.66 $mS \cdot cm^{-1}$) whilst in Lot 2 the salinity is medium with an EC of 1.53 $mS \cdot cm^{-1}$.

The organic matter (MO), as the main source of Nitrogen (N), is satisfactory in both Lots, but the content is higher in Lot 1.

Regarding to phosphorus (P), it is in the optimal range (17 ppm) in Lot 1 but excessive (80 ppm) in Lot 2. It seems that frequent application of fertilizers in Lot 2 has induced toward an increment of residual effects of P in the soil. Potassium (K) is excessive in both Lots as Calcium (Ca) and Magnesium (Mg) are.

Figure 1. Soil profile of a *Luvisol* at the Uxmal experimental station. Muna Yucatan, Mexico.

Table 1. Chemical attributes of Lot 1 and Lot 2. Uxmal experimental station.

Soil attributes	LOT		
	1	2	Reference from Official Mexican Norm.
pH	6.72	6.76	(6.6 - 7.3)
C.E. ($mS \cdot cm^{-1}$)	0.66	1.53	1.1 - 2.0 (Very lightly saline)
Na (ppm)	165	330	150
M.O. (%)	2.78	2.11	(1.6 - 3.5)
$N\text{-}NO_3$ (ppm)	14.8	17.2	(20 - 40)
P (ppm)	17	80	(15 - 30)
K (ppm)	1,365	1,170	(117 - 234)
Ca (ppm)	3,600	2,800	1000 - 2000
Mg (ppm)	1,000	920	156 - 360

The comparison of the results were made considering, as reference, the Nom-021-SEMARNAT-2002 [8].

The excessive contents of Ca, in the soil, are due to its intrinsic genetic formation. In these soils, the Calcium Carbonate ($CaCO_3$) is a dominant factor. During its dilution, Ca as an ion (Ca^{2+}) form released continuously to the soil solution. In that way, Ca can interfere in the plant absorption of other essential elements such as potassium (K) in an antagonistic process.

2.2. Reference Soil Values for Soil Fertility

The soil reference critical levels, for comparison purposes, were taken from the Official Mexican Standard that establishes specifications for fertility, salinity and soil classification, studies, sampling and analysis suggested (Table 1) by SEMARNAT, (2002) [9].

2.3. Treatments and Experimental Design

The free pollination Quality Protein Maize (QPM) varieties so called Sac Beh (*Sac*) and Chichen Iza (*Chich*) were the phytometers used. Both with white and yellow grain respectively.

Three treatments were studied as follow: 1) the Control (00-00-00), 2) the Chemical (*Chem*) fertilization with the formula (60-80-00) of N, P_20_5, K_2O where Potassium (K) was not applied due to the high levels in the soil, and 3) the Chemical fertilization with Bio-fertilizers (*Chem-Bio*) (60-80-00 + *Mycorrhizal fungi* + *Azospirillum bacteria*) expecting a good synergism between roots and bio-fertilizers.

The treatments were distributed in a completely random block design with three replications in experimental units of 5 m × 4 m (20 m^2) consisting of 4 rows of maize 5 m long, separated by 1.0 m and with distances of 0.40 m between strains of 2 plants in order to have an equivalent population density of 50,000 plants ha^{-1}.

The sowing was in the spring-summer of 2017 under well-distributed rainfed conditions. The unique chemical fertilizer was applied 15 days after planting, incorporated into the soil manually while the Bio-fertilizers were added to the seed at planting time.

2.4. Inoculation of Bio-Fertilizers and Chemical Fertilization

Bio-fertilizers were applied to the seeds (**Figure 2**) with a mixture (1:1 ratio) of both: 1) INIFAP™ brand biofertilizer with *Rhizophagus intraradices* (*Mycorrhizae* fungus) at a concentration of ≥60 spores and 2) *Azospirillum brasilense* (Bacterium) at a concentration of 1×10^{-6} Colony Forming Units (CFU) mL^{-1}. The seeds were mixed (**Figure 3**) with the Bio-fertilizers and dried at room temperature for 8 hours before planting to the experimental plots. The fertilizer was applied 15 days after sowing and buried 10 cm from the stem in the form of Urea (46% N) and Triple Calcium Superphosphate (46% P_2O_5) in a single application.

Figure 2. Applying Bio-fertilizers to seeds.

Figure 3. Seeds mixing with Bio-fertilizers.

2.5. Procedures for Taking Foliar Samples

For foliar sampling, five opposite leaves from the ears were taken at silk stage in each experimental unit. The five leaves, from the same number of plants randomly selected, were mixed together to make a composite sample to be sent to the laboratory. The samples were dried at 70˚C during 72 hours and the content of N, P, and K was determined in the laboratory [10] in percentage (%); and the critical levels were those reported by Jones and Eck (1973) [11] as reference values.

3. Results and Discussion

3.1. Nutritional Content of Nitrogen (N) in Leaves

In both lots, the two varieties showed N deficiencies in all treatments; however, in the case of *Sac* the general average of N from all treatments was higher in the Lot 1 (2.02%) than in the Lot 2 (1.81%). In contrast, the *Chich* variety, showed an opposite trend since the average N content was lower in Lot 1 (1.80%) than in Lot 2 (1.86%).

In the extreme cases like the *Control* and the high intensive soil use of Lot 2, it was observed that *Sac* had a higher N content (1.86%) than *Chich* (1.72%); suggesting a better efficiency of *Sac* to extract N from the soil and export it into the plant.

The N content in plants increased when chemical fertilizer (*Chem*) and its combination with bio-fertilizer (*Chem-Bio*) was applied as compared with the *Control*. However, this trend occurred only in *Sac* when it was planted in Lot 1 (**Table 2**) and only in *Chich* when planted in Lot 2 (**Table 3**).

The added *Bio* effect, is detected when contrasting the *Chem* treatment with

Table 2. Foliar content of N, P and K in Sac Beh and Chichen Itza maize with different treatments in a *rhodic Luvisol* with low intensive use (Lot 1).

Varieties	Treatments	Grain Yield ($t \cdot ha^{-1}$)	N Foliar (%)	P Foliar (%)	K Foliar (%)
Sac Beh	*Control*	5.84	1.86	0.21	1.60
	Chem	6.68	2.04	0.24	1.76
	Chem-Bio	6.74	2.17	0.28	1.93
	AVERAGE	6.42	2.02	0.24	1.76
Chichen Itza	*Control*	6.55	1.88	0.24	1.70
	Chem	6.34	1.81	0.25	1.76
	Chem-Bio	6.80	1.71	0.24	1.93
	AVERAGE	6.86	1.80	0.24	1.79
	Reference Values Jones and Eck (1973)		2.54	0.27	1.68

the *Chem-Bio* one. In *Sac,* the difference was 2.04% vs 2.17% (Lot 1) whilst in *Chich* the difference was 1.89% vs 1.99%.

The behavior of N deficiencies was according to Remache *et al*. [12] who mentioned that in most tropical soils, the main limiting nutrient is Nitrogen (N) followed by Phosphorus (P); N is the most relevant nutrient in crop nutrition and some physiological processes of maize depend on its availability. Accompanying nutrients, which ultimately tend to affect crop yield is another factor to be considered.

Another factor, to explain the differentiated result between both varieties is highlighted by Aguilar-Carpio *et al*. 2015 [13] when observing genotypic differences due to the effect of Bio-fertilizer and N on the production of dry matter (DM) and yield (GY).

It was determined that only the maize variety VS-535 presented the best agronomic efficiency of nitrogen with the application of Bio-fertilizer (nitrogen-fixing bacteria Azospirillum and Mycorrhizal fungi *Glomus* sp.); however, when nitrogen fertilization was reduced (80 $kg \cdot ha^{-1}$ N), the Agronomic Nitrogen Efficiency (ANE) was higher, which indicates the potential of the genotype in the assimilation of nitrogen [13] due to the bio-fertilizer [14].

On the other hand, it seems that the rate of N applied (60 $k \cdot ha^{-1}$) as UREA was not good enough to supply N to the plant since maize of all treatments had N deficiencies. Even with the application of Bio-fertilizers, in combination with the chemical fertilizers, the N deficiencies persisted.

Related to this study, it has been found [15] that application of N (180 $kg \cdot ha^{-1}$) and P (120 $kg \cdot ha^{-1}$) significantly increased fodder yield of maize. The N was a limiting nutrient factor and there was a positive interaction with P. The uptake of N increased by N at higher application rates and so was the biomass component. Nitrogen losses to the environment is highly reduced.

3.2. Nutritional Content of Phosphorus (P) in Leaves

The same trend, as N in Lot 1, was for P content in *Sac* since it increased from the *Chem* treatment (0.24%) until the *Chem-Bio* one (0.28%) as compared to the *Control* with 0.21% (**Table 2**). The added effect of *Bio* was only noticeable in *Sac* when the foliar P content (0.28%) was in the satisfactory reference value considered as 0.27% [11]. In the case of *Chich*, in Lot 1, no differences were noted between treatments, and the values varied very little from 0.24% to 0.25%, just below the optimal one of 0.27%. The effect of residual P of the soil was noticed in Lot 2, for both varieties.

With one exception, as in *Chem* applied to *Chich* in Lot 2, all treatments had P contents above the critical level of 0.28% but opposite to the findings in Lot 1, the *Control*, in both varieties, had higher P content than *Chem* and *Chem-Bio* (**Table 3**). This suggests that the higher the P in the soil the lower the plant response to chemical fertilizers and Bio-fertilizers will be.

However, the added effect of *Bio* was detected when comparing *Chem* vs

Table 3. Foliar content of N, P and K in Sac Beh and Chichen Itza maize with different treatments in a *rhodic Luvisol* with high intensive use (Lot 2).

Varieties	Treatments	Grain Yield ($t \cdot ha^{-1}$)	N Foliar (%)	P Foliar (%)	K Foliar (%)
Sac Beh	*Control*	4.77	1.86	0.34	1.86
	Chem	5.71	1.77	0.29	1.76
	Chem-Bio	4.82	1.82	0.31	1.80
	AVERAGE	5.10	1.81	0.31	1.80
Chichen Itza	*Control*	5.29	1.72	0.33	1.56
	Chem	6.09	1.89	0.24	1.76
	Chem-Bio	5.68	1.99	0.28	1.71
	AVERAGE	5.68	1.86	0.28	1.67
	Reference Values Jones and Eck (1973)		2.54	0.27	1.68

Chem-Bio in both varieties of Lot 2. The *Sac* had 0.29% in *Chem* and 0.31% in *Chem-Bio* whilst for *Chich* the Pcontent was 0.24% for *Chem* and 0.28% for *Chem-Bio*.

One of the most important functions of *Mycorrhizae* is to improve the absorption of phosphorus (P) in plants, especially in soils low in P [16]. However, even though in Lot 1 with high content of P in the soil (80 ppm) the Bio-fertilizers worked efficiently; indicating that residual P, due to constant fertilizer applications, can be activated and introduced in to the plant regardless of the variety. This is a typical trend in intensive agriculture of developing countries in recent decades due to high application rates of phosphate fertilizers [17] [18].

3.3. Nutritional Content of Potassium (K) in Leaves

In the soil analysis, prior to the establishment of the experiment, it was determined that the Potassium (K) in the soil is excessive in both experimental Lots (**Table 1**) with more than 1000 parts per million (ppm) while critical levels are 117 to 234 ppm.

No maize plant showed nutritional deficiencies of K in the *Chem* and *Chem-Bio* treatments since they were above the critical value of 1.70%. However, in the *Control* (00-00-00) both varieties showed deficiencies depending on the Lot. *Sac* showed deficiencies in Lot 1 (1.60%) but not in Lot 2 (1.86%) while *Chich* showed deficiencies in Lot 2 (1.56%) but not in Lot 1 (1.70%).

There is a clearly trend observed in Lot 1, about the positive influence of *Bio* in the absorption of native K in both varieties. In the *Chem* treatment, the *Sac* variety obtained 1.76% of K, while when applying *Chem-Bio* the K increased to 1.93%. The same happened to *Chich*. This positive response and activation of soil K is due to the influence of Chemical fertilizers to solubilize native K of the soil. It seems that the antagonistic effect of Calcium (Ca) and Magnesium (Mg) on K is neutralized by fertilizers.

The application of *Bio* encouraged K uptake since the content in the leaves generally exceeded the chemical treatment applied alone. Consequently, more attention are to be paid on the effects of nitrogenous fertilizers on the reactivation of K in the soil and the synergism it causes when applied with Bio-fertilizers.

The *Sac*, under extreme conditions of intensive soil use and null application of *Chem* and *Bio* (Lot 2) can be more efficient in absorbing K than *Chich*. This is noted (Table 3) by having a higher content of K (1.86%) as compared to that of *Chich* (1.56%).

3.4. Grain Yield (t·ha^{-1})

In Lot 1, no significant differences were found between treatments. Regardless of the treatments, the average yields of both genetic materials were higher in Lot 1 with the low agricultural use than in Lot 2.

The yield of *Sac* in Lot 1 was 6.42 t·ha^{-1} against that of Lot 2 with 5.10 t·ha^{-1}, a difference of 1.32 t/ha. Meanwhile, *Chich* obtained 6.86 t·ha^{-1}, in Lot 1, and 5.68 t·ha^{-1} in Lot 2. There was a general trend of yields associated with N in *Sac.* The-higher the yield (Lot 1) the higher the general average of N in leaves (2.02%) and the lower the yield (Lot 2) the lower the general average N (1.81%). This was not the case of *Chich* which showed a higher general average of N in Lot 2 (1.86%) than in Lot 1 (1.80%) but grain yields were in the opposite trend.

There was not a direct proportional relationship of foliar P and yields for any variety. The general average P content of *Sac* in Lot 2 (0.31%) was higher than in Lot 1 (0.24%) whilst for *Chich* the same trend happened with 0.28% in Lot 2 and 0.24% in Lot 1. The same behavior happened with K, whose contents in leaves do not present a defined tendency to be associated with yields.

4. Conclusions

Sac was more efficient in extracting N than *Chich* in the less intensively used soil (Lot 1) whilst *Chich* was more efficient in extracting N than *Sac* in the most intensively used soil (Lot 2).

The two varieties showed N contents in leaves below the critical range, in both experimental lots, even with the application of fertilizers.

In Lot 2, with more intensive soil use, both varieties showed sufficient foliar P contents including the control (00-00-00) due to the residual effects of fertilizers applied in the soil.

The *Sac*, under extreme conditions of intensive soil use and no fertilization, can be more efficient in absorbing K than *Chich*.

Practically both materials, in both lots, showed sufficient potassium (K) in the leaves due to the excessive native K in the soil.

Important findings support that native K of the soil can be more available to plants at the application of Chemical fertilizers (*Chem*) alone or combined with Bio-fertilizers (*Chem-Bio*) as compared to the *Control* with no *Chem* nor *Bio.*

The results here obtained, need more details of information on different agro-ecological conditions of soils, climates and crops. It is expected different crop responses due to sources, quantities, and time of application of any organic and chemical material.

Acknowledgements

We thank the National Institute of Forestry, Agricultural and Livestock Research (INIFAP) of Mexico for financing this work as part of the project called: Eficiencia nutrimental con fertilización química y orgánica en *Luvisoles rodicos* de Yucatán (Nutrient efficiency with chemical and organic fertilization in *rhodic Luvisols* of Yucatan).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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