

#### **FINAL REPORT**

Evaluation of the Biological Effectiveness of "EL ORGULLO DE MÉXICO" Fertilizer on Sugarcane

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Date of Preparation: 14-02-25

This report describes the results observed after applying various treatments of the fertilizer "Orgullo de México" to sugarcane, in accordance with the protocol previously established in accordance with NOM-077-FITO-2000, which establishes the specifications, criteria and procedures for evaluating biological effectiveness studies of plant nutrition inputs. The main variables of agroindustrial performance of sugarcane crops will be considered.

#### **MATERIALS AND METHODS**

Area of study. The evaluation was carried out in an area of 5400 m2. The experiment was established in commercial crop areas with conventional agronomic management (nutrition and phytosanitary) in the town of Potrero Viejo in the sugarcane region of Córdoba-Golfo, Veracruz. A crop with new sowing was considered.

Treatment design. The experiment was conducted with a completely randomized block arrangement with four replicates per treatment. The treatments considered were the single application of organic fertilizer "El Orgullo de México", biweekly applications, monthly application of the fertilizer and the absolute control (Table 1).

Table 1. Design of treatments to be evaluated

Treatment	Description of treatments
T1	Absolute witness
T2	Single application of organic fertilizer "El Orgullo de México" at a rate of 40L/ha
Т3	Triple application, every 15 days of organic fertilizer "El Orgullo de México" at a rate of 40L/ha
T4	Triple application, every 30 days of organic fertilizer "El Orgullo de México" at a rate of 40L/ha

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#### **RESPONSE VARIABLES**

While the evaluations of vegetative development have been carried out so far at 15, 30, 60 and 90 days after the start of the experiment. The determinations of the response variables will be carried out considering three replicas or experimental units per treatment. Therefore, it must be considered that each repetition will consist of experimental units that will be the strains within each block (Table 2).

Table 2. Response variables in the evaluation of the "Pride of Mexico" fertilizer

F	Response Variable	Description
	(RV)	Description
	Emergence or	15 and 30 days after application.
1	germination	Stems that show signs or presence of true leaf blades will
	germination	be considered as germinated.
		30, 60, 90 and 120 days after application.
2	Number of stems	All stems per strain will be considered in a 10 m transect
_		with three replicates per block.
		The average number of stems per strain will be calculated.
3	Stem thickness	60 and 90 days after application.
4	Height per stem	Three random stems will be considered per strain with three
	<u> </u>	replicates per block.
5	Leaf blade length	The average thickness per stem will be calculated.

Physicochemical diagnosis of soil. A physical-chemical analysis was performed prior to the application of the treatments, in order to identify the state of the substrate in which the evaluation of the "Orgullo de México" fertilizer was to be established. The soil sampling and analysis was carried out under the method established in the Mexican Official Standard 021-RECNAT-2000, which establishes the specifications for fertility, salinity and soil classification, studies, sampling and analysis with application throughout the national territory.

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Application of treatments. The application of general treatments was carried out on October 20, 2024 under the conditions established in the protocol presented above, with manual application using motorized spraying equipment with a hollow cone nozzle, at the bottom of the furrow before the sugarcane seed was covered with soil (Figure 1).



Figure 1. Application of treatments. a) and b) dosage of treatments; c) application of treatments and d) Covering of sugarcane seed with a yoke.

While the subsequent application of treatments was carried out under the established protocol, recording the climatological variables on each occasion (Table 3).

Application	Climatic variables				
Application	Temperature (°C)	Humidity (%)	Precipitation (mm3)	Radiation	
10/20/24	26	59	0.25	Low	
5/11/24	25	32	1	Low	
11/19/24	25	30	0	Low	
03/12/24	24	59	1	Low	
12/20/24	23	63	0	Low	

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Application of subsequent treatments. The application of general treatments was carried out on October 20, 2024 under the conditions established in the protocol presented above, with manual application using motorized spraying equipment with a hollow cone nozzle, at the bottom of the furrow before the sugarcane seed was covered with soil (Figure 2).





Figure 2. Application of treatments.

Regarding the rest of the treatments, these were carried out gradually every 15 days according to the schedule established in the initial protocol. A total of six applications were made for the T3 treatment, four applications for T4 and one application for T2. The applications were made with manual equipment and directed to the soil or the base of the strain, during the morning between 7 and 8.

The complete photographic material was attached as evidence in folders per application uploaded to Google Drive

(https://drive.google.com/drive/folders/1XYMeFwYvcfW6XHQO1Lb7EA 1hf5ZiTzdN? usp=drive\_link).

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#### **RESULTS**

Physicochemical diagnosis of soil. The results indicated that the soil where the evaluation was established is a healthy substrate with a neutral pH, which guarantees that the applied treatments will be used by the plant without complications.

This is a soil with a clayey to clayey loam texture class, ideal for growing sugar cane, with a normal percentage of organic material, which is clearly influenced by the application of treatments, with applications every 30 days being the treatment that reaches the highest percentage of 9.02%. Soil free of carbonates and salts, which guarantees a substrate with the capacity to receive and assimilate nutrients. Very high supply of available phosphorus (Table 3).

Table 3. Soil diagnosis determinations and interpretation.

Variable	No crop	Absolute witness	Single Application	15-day application	30-day ap	oplication	Optimal level for cultivation
pН	6.56	6.61	6.02	6.52	6.48	Neutral	5.5- 7.8
Textural Class	Clay loam	Clay loam	Clay loam	Clay loam	Cla	ay	
MO	4.3	5.93	6.81	8.53	9.02	very high	<4 mg/kg
Saturation Point	48	18.4	18.1	61.6	60	%	30%
Field Capability	25.6	31	30.5	33	32.1	%	20%
Cond. Hydraulics	2.2	0.9	1.1	0.8	0.8	cm/hr	5cm/hr
Bulk density	1.31	1	1.11	1.02	1.01	1 g/cm3	
CE	0.3	1.01	1.13	1.33	1.17	dS/m	<1
N-NO3	4.44	52.3	53	74.2	54.5	mg/kg	10-60 mg/kg
P	130	143	157	568	334	mg/kg	10 mg/kg
K	372	341	442	435	402	mg/kg	78 mg/kg
Fe	28.2	18.1	23.2	48.4	32.2	mg/kg	50-250 mg/kg
Cu	1.18	1.05	1.14	1.45	1.05	mg/kg	1 a 3 mg/kg
В	0.1	1.68	1.7	1.72	1.68	mg/kg	0.4 a 0.6 mg/kg
S	19	5.18	5.18	10.4	9.07	mg/kg	78 mg/kg
Mg	217	181	218	297	252	mg/kg	20-40 mg/kg
Zn	2.45	2.54	3.45	4.45	4.36	mg/kg	1.5 a 4 mg/kg
Mn	37.2	18.6	28.6	22	13.5	mg/kg	20-40 mg/kg
Ca	1799	2309	1683	2457	1784	mg/kg	1500 mg/kg
Na*	25	25	25	25	25	mg/kg	<25
Al*		0	0.21	0	0.21	mg/kg	<2

<sup>\*</sup>It is desirable that these elements have a low content
\*\*variables marked in green present the highest content

Regarding the rest of the parameters, they are influenced by the application of the treatments, with the soil treated with applications every 15 days being the one that reached the highest nutritional levels. Regarding Aluminum, it was only possible to determine it in two treatments in a single application and every 15 days, but with very low levels almost imperceptible by the plant, so it is considered that the effect of this element will even be positive for the development of the plant.

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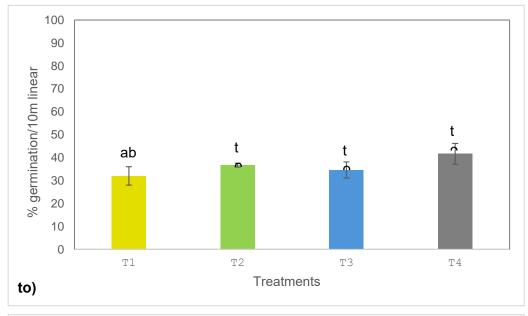
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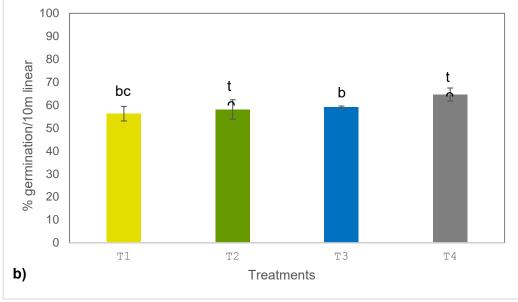
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In general, a soil with a high nutrient content and capacity for nutrient absorption. Without problems in its physical characteristics that allow for ideal moisture drainage.

Emergence or germination. Sugarcane seed germination was determined at 15 and 30 DAA, observing an increase in the percentage of germinated buds of 24.47% in the buds treated with T3. While the highest percentage of germinated buds was observed in the sites treated with T4, whose results were 64.58% germination (Figure 3).

\*Equal letters indicate the absence of significant differences between the treatments studied.





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Figure 3. Percentage of sugarcane bud germination per 10 linear meters. a) Determination made at 15 DAA. b) Determination made at 30 DAA.

Regarding the germination percentage of sugarcane buds, since the recorded data did not follow a normal distribution, a Kruskal-Wallis test was performed to identify the existence of statistical differences between the sites applied with the different treatments and a Games-Howell multiple comparison test. The analyses were performed with a significance value of  $\alpha$  < 0.05, in the statistical package version RStudio version 3.6 for Windows (R Core Team 2018).

It was determined that until the application carried out on December 3, 2024, there were no statistically significant differences between the applied treatments (Letters located at the top of the bars of the graph indicate the absence of significant differences between the treatments studied) (P=0.002; df =14).

However, in the study, the buds treated with T3 and T4 showed the highest percentages of germination, so it could be considered that both treatments have a positive effect on the germination of sugarcane seeds and could even be attributed an effect as an inducer of bud germination (Figure 4). Although as can be observed in Figure 3, the bar for T3 shows a smaller range of variation in its results (smaller error bar), which is an indication that the germination of buds with this treatment was more homogeneous than even in T4. Figure 4. Germinated buds at 15 DAA. a) Germination of linear buds T3. b) Germination of linear buds T4





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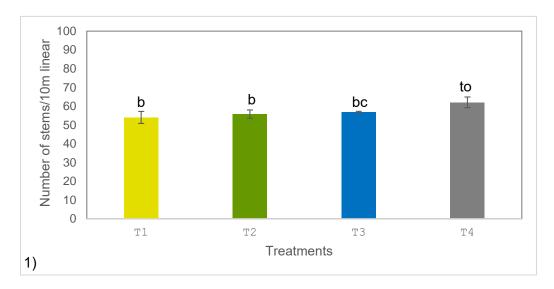
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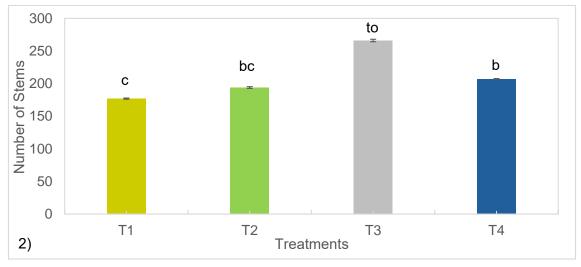
Number of stems. This variable was determined at 30, 60 and 90 DAA, observing that the highest number of stems with true leaves was in T4 with 12.9 true stems/strain. A Kruskal-Wallis test was performed, which allowed identifying the existence of statistical differences between the sites applied with the different treatments and a Games-Howell multiple comparison test in the three measurements. The analyses were carried out with a significance value of  $\alpha$  < 0.05, in the statistical package version RStudio version 3.6 for Windows (R Core Team 2018).

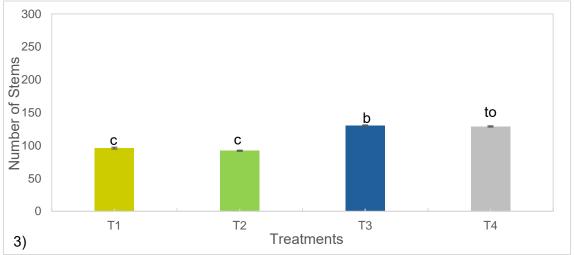
It was possible to identify that there are statistically significant differences between the treatments ( $P \le .001$ ; df = 14,  $P \le .001$ ; df = 19,  $P \le .001$ ; df = 19, respectively for the 30, 60 and 90 DDA measurements), a maximum in the number of real stems of 130 in T3 which also presents a similar behavior to the previous variable with data of greater homogeneity (smaller error bar) as can be seen in the blue bar in Figure 5, although with a lower number of real stems/10 linear m.



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\*Equal letters indicate the absence of significant differences between the treatments studied. Figure 5. Number of true sugarcane stems per 10 linear m. 1) Determination at 30 DAA, 2) Determination at 60 DAA, 3) Determination at 90 DAA.

It is possible to observe a high number of real, turgid and constantly growing stems, and because the stems presented in the last evaluation already present phenological differentiation in the formation of nodes and internodes, most of them will move on to the maturity stage, considering a loss of around 10% of stems due to environmental, nutritional and phytosanitary conditions (Table 2).

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Table 2. Theoretical estimation of DDA field performance.

Treatment	Number of stems/10m2	Number of stems/ha	Yield estimate (1 stem = 980g)
T1	96	96,000	94.08 tons/ha
T2	92	92,000	90,160 tons/ha
Т3	130	130,000	127.4 tons/ha
T4	129	129,000	126.4 tons/ha



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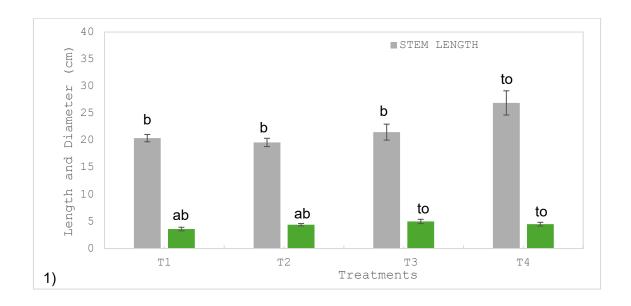
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Figure 6. True stems at 30 DAA. a) and b) linear stems at 30 DAA. c) and d) linear stems at 90 DAA.

Length and diameter of stems. With respect to this variable, determinations were made at 60 and 90 DAA, and it was possible to observe that there is a positive effect on the crop with the application of the treatments, especially in those areas that were applied constantly (T3 and T4).

It was possible to identify that there are statistically significant differences between the treatments for the stem length case ( $P \le .001$ ; df = 22,  $P \le .001$ ; df = 19, respectively for the measurements), an average of 26.9cm in the stem length variable for the T4 treatment. While for the stem diameter variable the greatest thickness was in the T3 treatment (5cm), with stems 0.5cm larger in diameter than those present in the areas treated with T4.

However, no significant differences are observed between treatments T3 and T4, so the practice of selecting any of the two treatments would have similar results, but superior to the rest of the treatments applied, including the control. It is important to mention that in the case of sugar cane, robust stems are preferable because it is inferred that there is a higher concentration of sucrose at the maturity stage and a greater possibility of survival (Figure 7).



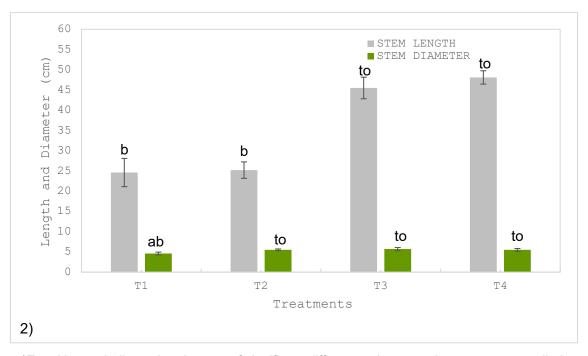
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\*Equal letters indicate the absence of significant differences between the treatments studied. Figure 7. Stem length and diameter determinations in 10 linear meters. 1) Determination at 60 DAA, 2) Determination at 90 DAA.

Regarding the measurements, a photographic evidence is attached, which is representative of the dimensions of the observed stems (Figure 8). The complete photographic material can be found in the Drive folder.



Figure 8. Determination of stem length and diameter at 90 DAA for T4

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#### **CONCLUSIONS**

- The findings of this evaluation allowed us to observe a positive effect on the applied areas in all the response variables studied.
- In relation to the nutritional status of the soil after the application of the treatments, an increase in important variables such as organic matter, N, P, K was induced, so according to what was observed, the treatment with the greatest effect was T4. While T3 generates a greater increase in some elements, T4 has a greater effect on organic matter, which guarantees the use of nutrients by the plant.
- Regarding the germination variable T4, it presents the greatest effect, which guarantees the success of sowing and application of the product.
- While the variables length and diameter present the greatest effects in treatments
   T3 and T4.
- Therefore, for practicality and investment, it is recommended to apply the organic product Orgullo de México monthly, considering a minimum of 2 to 3 applications from the beginning of the crop until the stage of great growth.