EVALUATION OF BIOLOGICAL EFFECTIVENESS OF THE PRODUCT PROTECSEM (BIO SEED IN USA) AS INOCULANT, IN CORN CROP, CARRIED OUT IN THE MUNICIPALITY OF COJUMATLÁN DE REGULES, MICHOACÁN.

NAME, CURP AND ADDRESS OF THE PERSON IN CHARGE OF THE STUDY:

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INTERESTED COMPANY: **QUÍMICA LUCAVA, SA de CV** Carretera Panamericana, Km 284, 2da. Fracción de Crespo, CP 38110, Celaya, Gto. Mexico .

a) Institution that will carry out the Biological Effectiveness study.

Autonomous University of Aguascalientes. Jesús María Agricultural Sciences Center, Aguascalientes, Mexico.

b) Type of Input: Inoculant

c) Report Title:

Evaluation of biological effectiveness of the product Protecsem (Bio Seed in USA) as inoculant, in corn crop, carried out in the municipality of Cojumatlán de Regules, Michoacán.

d) Introduction

1. Importance of the crop

Corn is the second most important crop in the world for its production, after wheat, while rice occupies the third place. It is the first cereal in grain yield per hectare and is the second, after wheat, in total production. Corn is of great economic importance worldwide either as human food, as feed for livestock or as a source of a large number of industrial products. The diversity of the environments under which corn is grown is much greater than that of any other crop. Having originated and evolved in the tropical zone as a plant with excellent yields and its post-harvest period is longer and when stored it is destined for human and animal consumption (FAO, 2001).

Mexico is considered as its center of origin and diversity of different strains, each with different domestic and wild varieties (Muñetón , 2009). Based on the numerous products and applications obtained from corn, it is considered to be of great economic and nutritional value, being the support of Mexican families, either for self-consumption or for sale, also having a social and cultural impact (Kato *et al. al.,* 2009).

1.1. Production in Mexico

Corn production in Mexico is approximately 16,164,603.31 MT; The main producer of corn in Mexico is the state of Jalisco, which produces 4,663,959.23 MT per year equivalent to 28.85% of the national production, followed by the state of Durango with a production of 2,409,416.42 MT, that correspond to 14.90%; the state of Zacatecas produces 14.04% with a production of 2,269,739.19 MT (Siap , 2016).

e) Objetives:

1. Evaluate the biological effectiveness of the product PROTECSEM (BIO SEED in USA), as an inoculant in corn crop.

2. Determine the possible phytotoxic effects of the product PROTECSEM (BIO SEED in USA), as an inoculant in corn crop.

f) Commercial and / or experimental name.

PROTECSEM (BIO SEED in USA)

g) Guaranteed Composition:

Guaranteed Composition	Concentration
Paenibacillus azotofixans	1 x 10^8 CFU/g
Bacillus megaterium	1 x 10^8 CFU/g
Bacillus mucilaginosus	1 x 10^8 CFU/g
Bacillus subtilis	1 x 10^8 CFU/g
Trichoderma harzianum	1 x 10^8 CFU/g

CFU: Colony forming units

MATERIALS AND METHODS

LOCATION OF THE EXPERIMENTAL SITE.

The study was installed in a commercial corn plot in the municipality of Cojumatlán de Regules, Michoacán. The coordinates of the place where the study was established are 20.62556 N,-102.894972 W.

h) Start date of the study: April 24, 2021

i) Study completion date: October 3, 2021

j) Crop in which the study was carried out:

Corn, Variety: DK 4018

k) Phenological (growth) stage of the plant:

Sowing, growth and production stages

l) Trial layout

1. The trial was established under a Latin Square layout, with four rows and four columns.
2. The experimental unit (plot) was made up of 4 rows at a distance between rows of 0.8 m, giving 3.2 m width and 5 m length, in total 16 m^2, that is, 64 m^2 per treatment. Therefore, a total area for the study of 256 m^2 was used.
3. During the evaluations, 0.5 m between treatments and a row on each side were discarded , leaving a useful (evaluation) plot of 2 rows of 0.8 m width by 4.0 m length, this is, 6.4 m^2.

m) Distribution of treatments

The distribution of treatments in the field after randomization was as follows.

Table 1 . Distribution of treatments in the field:

	COLUMN I	COLUMN II	COLUMN III	COLUMN IV
ROW IV	T4	T3	T2	T1
ROW III	T3	T2	T1	T4
ROW II	T2	T1	T4	T3
ROW I	T1	T4	T3	T2

Arabic numerals = Treatments

n) Rate, timing and number of applications

The treatments that were evaluated are indicated in Table 2.

Table 2 . Treatments of PROTECSEM as inoculant in corn crop.

Tr.	Product	Rate g/ha	Rate g per plot (16 m^2)	Rate g per treatment (64 m^2)	Water ml/ treatment @ 7 ml / g Protecsem
1	Untreated control				
2	PROTECSEM	90	0.144	0.576	4
3	PROTECSEM	125	0.200	0.800	6
4	PROTECSEM	180	0.288	1.152	8

Planting density was 90,000 plants/ha, weighing 26,235 kg (60,000 seeds weigh17.49 kg), equivalent to 576 plants & 0.168 g of seed per treatment, 144 plants & 0.042 g of seed per plot.

o) Timing and number of applications

A single application in seed treatment at planting was carried out.

Application method: seed treatment.

Application equipment: seed treatment in 1 L capacity jar.

Volume of water used:

Seed treatment: A slurry was previously prepared at the rate of 7 ml of water per g of Protecsem and the seed was covered well with it.

p) Other inputs used in the evaluation:

No other type of input was used in this trial,

q) Variables for estimating biological effectiveness and evaluation method.

1. Phytotoxicity . Assessed at 7 and 14 days after planting by the percentage scale proposed by the European Weed Research Society (Table 3).

Table 3 .Percentage scale by the European Weed Research Society, to evaluate the possible phytotoxic effect of the product PROTECSEM in corn CROP.

EFFECTS ON CROP	Crop Phytotoxicity (%)
No effect on crop	0.0-1.0
Very mild symptoms	1.1-3.5
Mild symptoms	3.6-7.0
Moderate symptoms, but without effect of yields	7.1-12.5**
Intermediate damage	12.6-20.0
High damage	20.1-30.0
Very high damage	30.1-50.0
Extremely high damage	50.1-99.0
Complete destruction of crop	99.1-100

Transformation of the logarithmic scale of EWRS to a percentage scale. ** Limit of acceptability.

2. **Emergence in trays (laboratory):** 100 seeds per replicate, i.e. 400 seeds per treatment, were placed to germinate in the laboratory.

3. **Emergence in the field:** at 7 and 14 days after planting, the emergence % per meter was measured.
4. **Root fresh and dry weight:** 3 plants were taken per experimental unit (plot) at 14 days after sowing and the fresh and dry weight of roots were taken.
5. **Root length:** 3 plants were taken per experimental unit (plot) at 14 days after sowing and the length of the root was measured.
6. **Stem diameter (mm):** stem diameter was measured with a vernier in 3 plants at random per plot, at 60 days after planting. The results are reported in mm.
7. **Plant height (cm):** was measured with a measuring tape in 3 plants at random per plot, at 60 days after planting. The results were reported in cm.
8. **Chlorophyll content in leaves**. Two leaves were taken in three plants per plot, which were measured with the SPAD method, which determines the relative amount of chlorophyll present, through the measurement of leaf absorption in two wavelength regions: in the red and near infrared regions. Using these two transmissions, the meter calculates the SPAD numerical value, which is proportional to the amount of chlorophyll present in the leaf and consequently of nitrogen, at 60 days after planting.
9. **Fresh weight of the plant.** 3 plants were taken per plot and weighted in a scale at 60 days after planting.
10. **Dry weight of the plant.** 3 plants were taken per plot and weighted in a scale at 60 days after planting.
11. **Leafless Fresh ear weight:** 5 ears were taken per plot and weighed.
12. **Number of ears per plant:** 5 plants were taken per experimental unit (plot) and the number of ears was counted .
13. **Yield ($MT.ha^{-1}$):** The ears were harvested and weighed per linear meter and per experimental unit (plot) and the yield was obtained .

r) Evaluation method to allow a statistical analysis, according to the trial layout.

ANALYSIS OF DATA. From the data obtained of the variables: emergence in trays and in the field, fresh and dry weight of the root, root length, stem diameter, plant height, chlorophyll content, fresh and dry weight of the plant, fresh weight of ears, ear number and yield were statistically analyzed by analysis of variance and mean comparison test of Tukey ($\alpha = 0.05$), using the statistical package SAS.

s) Sample size and sampling method. The sample size was previously specified for each variable.

t) CALENDAR OF ACTIVITIES. It is shown in Table **4.**

Table 4 . Calendar of evaluation activities of biological effectiveness of Protecsem in corn crop.

ACTIVITY	DATE
Seed treatment and planting	April 24, 2021
Germination evaluations @ 7 & 14 days after planting (dap)	May 1 & 8, 2021
Phytotoxicity evaluations (14 & 30 dap)	May 8 & 24, 2021
Growth & phyto variable evaluation (60 dap)	June 23, 2021
Harvest variable evaluation (160 dap)	October 3, 2021

dap. Days after planting

RESULTS AND DISCUSSION

1. Emergence or germination y trays (%)

The analysis of variance of the **% of emergence or germination in trays** data in corn crop showed no significant difference between treatments. This was corroborated by carrying out a Tukey comparison of means ($\alpha = 0.05$).

Table 5 . Evaluation of the emergence or germination in trays in the corn crop .

TREATMENTS	% of emergence
T1. Untreated control (“Testigo absoluto” in Figure)	99.8 A
T2. PROTECSEM (90 g.ha^{-1})	99.8 A
T3. PROTECSEM (125 g.ha^{-1})	99.5 A
T4. PROTECSEM (180 g.ha^{-1})	100.0 A

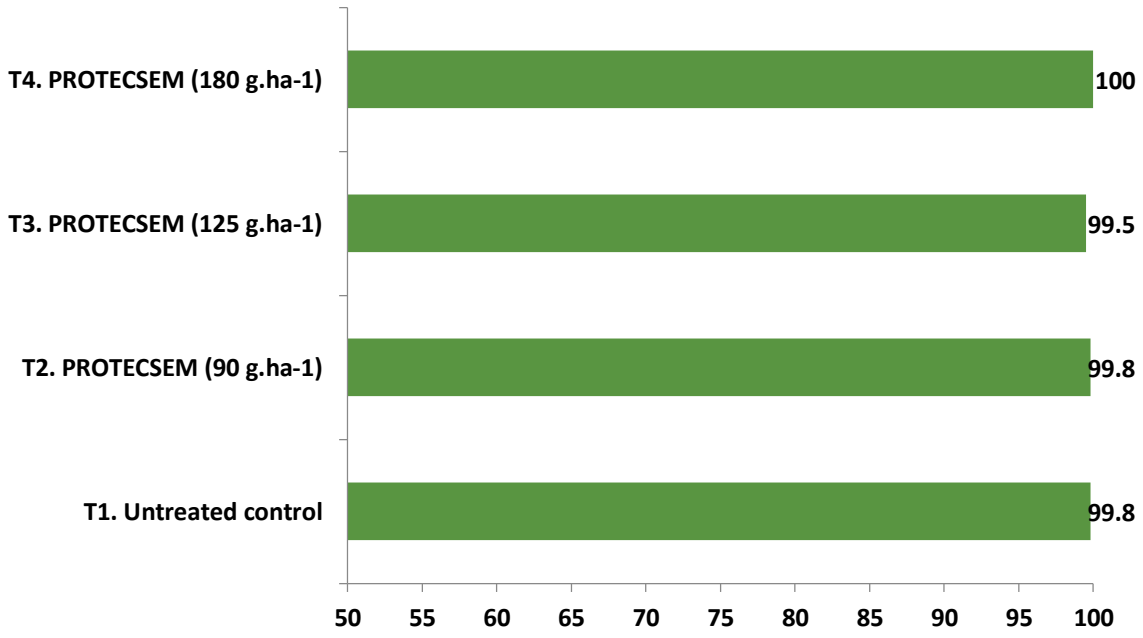

Figure 1. Emergence or germination in trays (%)

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2. % Emergence or germination in the field

The analysis of variance performed of **emergence (germination) in field** data in corn crop showed no significant differences between treatments evaluated. This was corroborated by carrying out a Tukey comparison of means ($\alpha = 0.05$).

Table 6 . Evaluation of the emergence variable in the field (%) **,** in the corn crop.

TREATMENTS	% field emergence	
	7 dap	**14 dap**
T1. Untreated control ("Testigo absoluto" in Figure)	25.0 A	91.7 A
T2. PROTECSEM (90 g.ha^{-1})	22.2 A	94.4 A
T3. PROTECSEM (125 g.ha^{-1})	19.4 A	97.2 A
T4. PROTECSEM (180 g.ha^{-1})	38.9 A	94.4 A

dap: days after planting

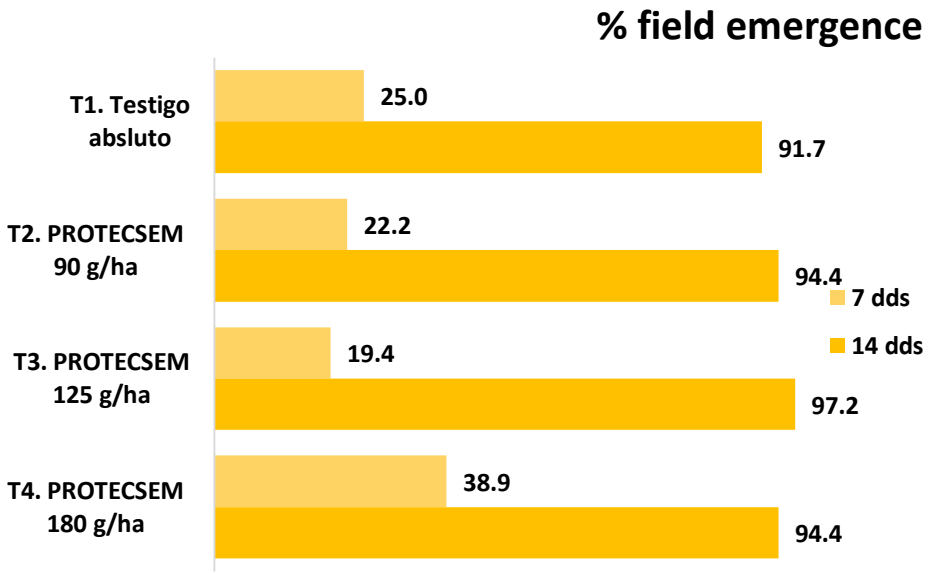

Figure 2 . Field emergence (%) at 7 and 14 dap.

3. Fresh root weight

In the analysis of variance performed with the fresh root weight data in corn crop, significant differences were observed compared to the Untreated control and between treatments, at 14 days after planting. This was corroborated by conducting a Tukey comparison of means (α=0.05).

It was observed that the fresh weight of root was higher where PROTECSEM was applied at 125 g.ha^{-1}, with a mean of **0.35 g**, and although without significant differences, numerically was also greater where Protecsem was applied at 180 g.ha^{-1}, with a mean of **0.32 g**, compared to the control that showed a mean of **0.22 g**, (Table 7) (Figure 3).

Table 7 . Evaluation of the **fresh root weight** variable in the corn crop

TREATMENTS	FRW
	14 dap
T1. Untreated control ("Testigo absoluto" in Figure)	0.22 B
T2. PROTECSEM (90 g.ha^{-1})	0.24 AB
T3. PROTECSEM (125 g.ha^{-1})	0.35 A
T4. PROTECSEM (180 g.ha^{-1})	0.32 AB

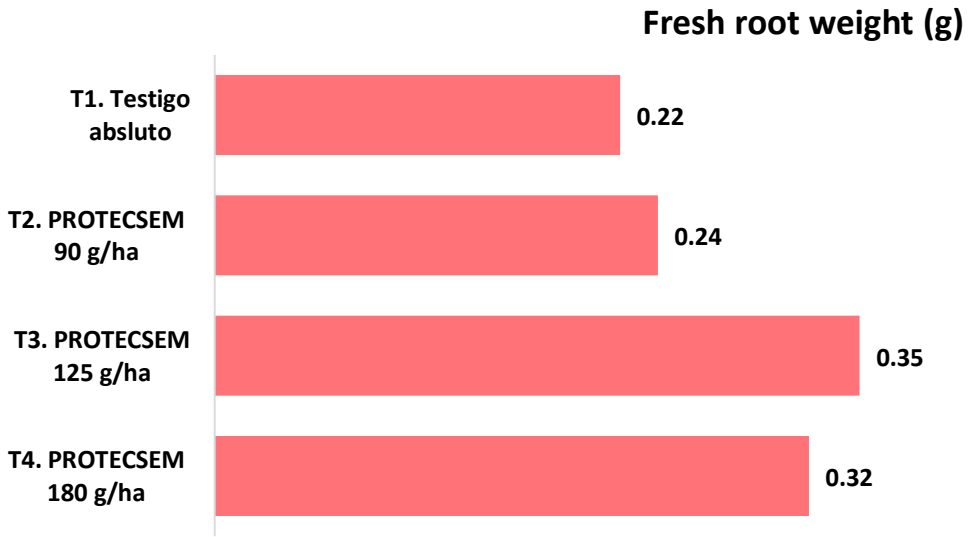

Figure 3 Root fresh weight.

4. Dry root weight

In the analysis of variance performed with the data of dry root weight in the corn crop, no significant differences were observed compared to the Untreated control, at 14 days after planting and application. This was corroborated by Tukey comparison of means (α=0.05). However, without significant differences, higher dry root weights were numerically observed in treatments of Protecsem 125 and 180 g.ha^{-1} than in the control.

Table 8 . Evaluation of the variable **root dry weight (g),** in corn crop.

TREATMENTS	DRW
	14 dap
T1. Untreated control ("Testigo absoluto" in Figure)	0.05 A
T2. PROTECSEM (90 g.ha^{-1})	0.05 A
T3. PROTECSEM (125 g.ha^{-1})	0.07 A
T4. PROTECSEM (180 g.ha^{-1})	0.06 A

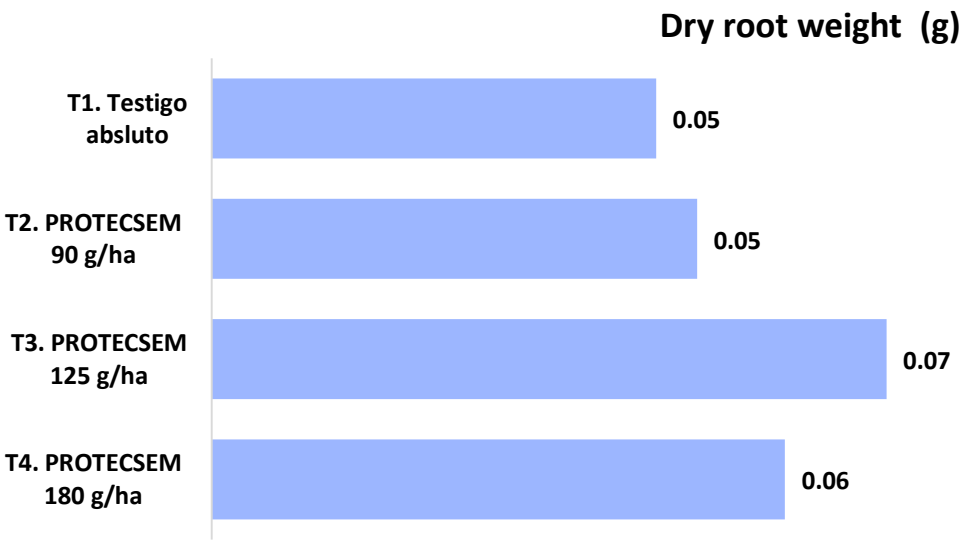

Figure 4 . Root dry weight

5. Root length

In the analysis of variance performed with the root length data in the corn crop, significant differences were observed compared to the Untreated control and between treatments at 14 days after planting. This was corroborated by a Tukey comparison of means (α=0.05).

It was shown that the root length was greater where PROTECSEM was applied at 125 $g.ha^{-1}$, with a mean of **97.2 mm**, and where it was applied at 180 $g.ha^{-1}$, with a mean of **94.6 mm**, followed without statistical difference by PROTECSEM at 90 $g.ha^{-1}$, with **84.9 mm**, compared to the control that showed a mean of **75.8 mm,** (Table 9) (Figure 5).

Table 9. Evaluation of the variable **root length (mm)** in corn crop.

TREATMENTS	RL
	14 dap
T1. Untreated control ("Testigo absoluto" in Figure)	75.8 C
T2. PROTECSEM (90 $g.ha^{-1}$)	84.9 BC
T3. PROTECSEM (125 $g.ha^{-1}$)	97.2 A
T4. PROTECSEM (180 $g.ha^{-1}$)	94.6 AB

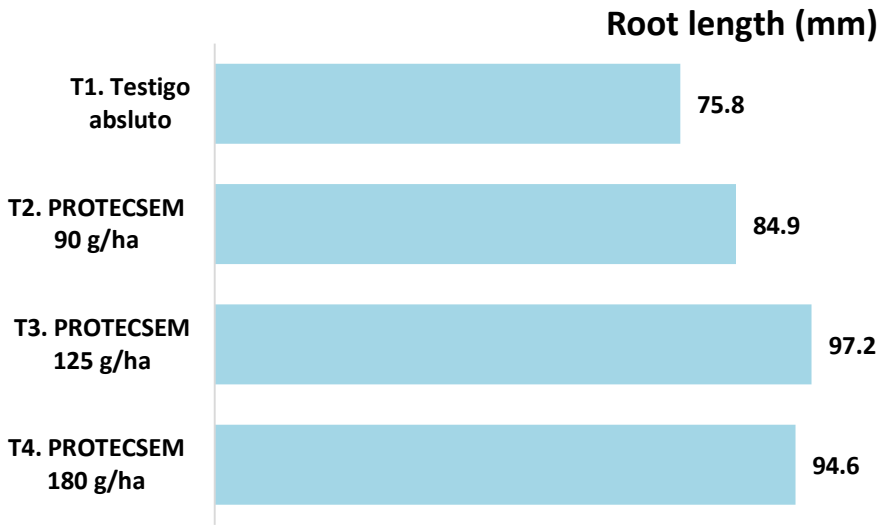

Figure 5 . Root length.

6. Stem diameter

In the analysis of variance performed with the data of stem diameter in the corn crop, no significant differences were observed compared to the Untreated control at 60 days after planting. This was corroborated by a Tukey comparison of means (α=0.05).

However, numerically, in all rates of Protecsem, larger stem diameters were observed compared to the control.

Table 10 . Evaluation of the variable **stem diameter**, in corn crop.

TREATMENTS	Stem diameter (mm)
	60 dap
T1. Untreated control (“Testigo absoluto” in Figure)	7.4 A
T2. PROTECSEM (90 $g.ha^{-1}$)	8.2 A
T3. PROTECSEM (125 $g.ha^{-1}$)	8.3 A
T4. PROTECSEM (180 $g.ha^{-1}$)	8.1 A

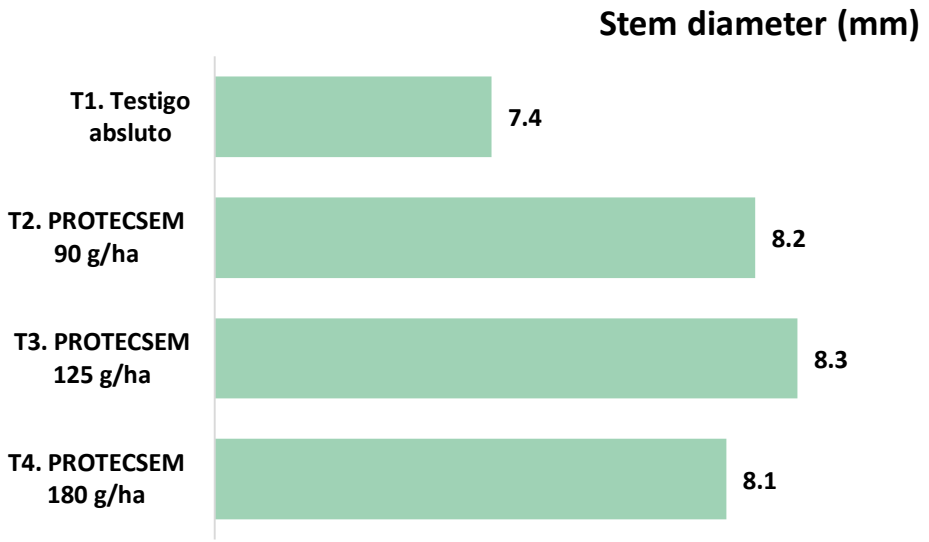

Figure 6. Stem diameter

7. Plant height

In the analysis of variance performed with the data of plant height in the corn crop, no significant differences were observed compared to the Untreated control at 60 days after planting and application. This was corroborated by a Tukey comparison of means (α=0.05).

However, numerically, in all rates of Protecsem, larger plant heights were observed compared to the control.

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Table 11 . Evaluation of the variable **height of the plant ,** in the corn crop

TREATMENTS	Plant height
	30 dap
T1. Untreated control (“Testigo absoluto” in Figure)	23.3 A
T2. PROTECSEM (90 $g.ha^{-1}$)	25.7 A
T3. PROTECSEM (125 $g.ha^{-1}$)	27.8 A
T4. PROTECSEM (180 $g.ha^{-1}$)	26.4 A

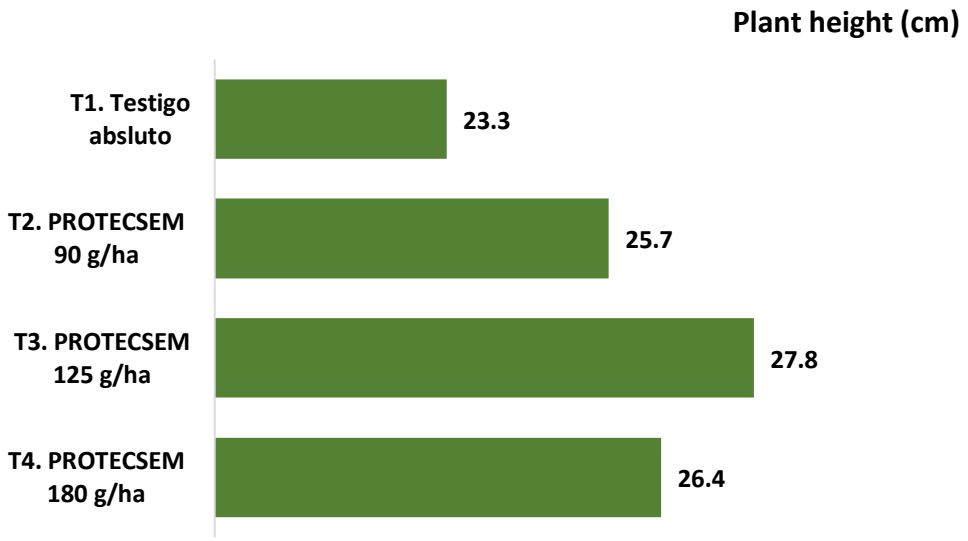

Figura 7. Plant height.

8. Chlorophyll content

The analysis of variance performed with the chlorophyll content data in corn crop, no significant differences were observed compared to the Untreated control at 60 days after planting and application. This was corroborated by a Tukey comparison of means (α=0.05).

Table 12 . Evaluation of the variable leaf **chlorophyll content (SPAD)** in corn crop

TREATMENTS	SPAD
	60 dap
T1. Untreated control (“Testigo absoluto” in Figure)	35.8 A
T2. PROTECSEM (90 $g.ha^{-1}$)	35.0 A
T3. PROTECSEM (125 $g.ha^{-1}$)	35.0 A
T4. PROTECSEM (180 $g.ha^{-1}$)	33.5 A

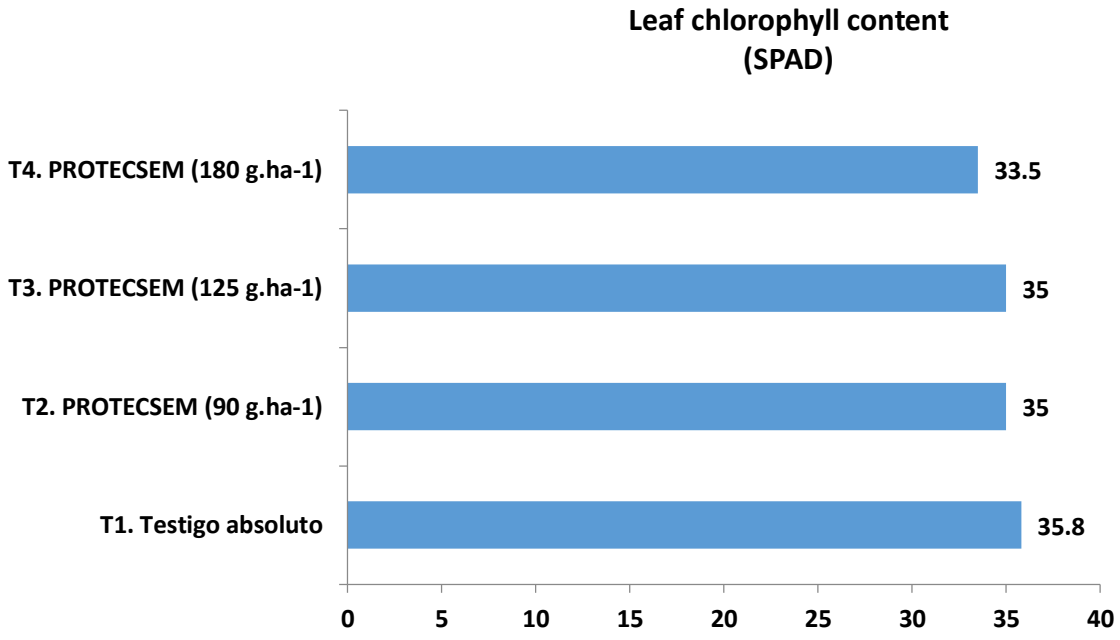

Figure 8. Chlorophyll content

9. Plant fresh weight

In the analysis of variance performed with the data of plant fresh weight in corn crop, no significant differences were observed compared to the Untreated control, at 60 days after planting and application. This was corroborated by a Tukey comparison of means (α=0.05). However, numerically higher plant fresh weights were observed in all the Protecsem treatments, compared to the control.

Table 13 . Evaluation of p**lant fresh weight** (kg) in corn crop.

TREATMENTS	Plant fresh wt
	60 dap
T1. Untreated control ("Testigo absoluto" in Figure)	15.8 A
T2. PROTECSEM (90 $g.ha^{-1}$)	17.5 A
T3. PROTECSEM (125 $g.ha^{-1}$)	23.3 A
T4. PROTECSEM (180 $g.ha^{-1}$)	20.8 A

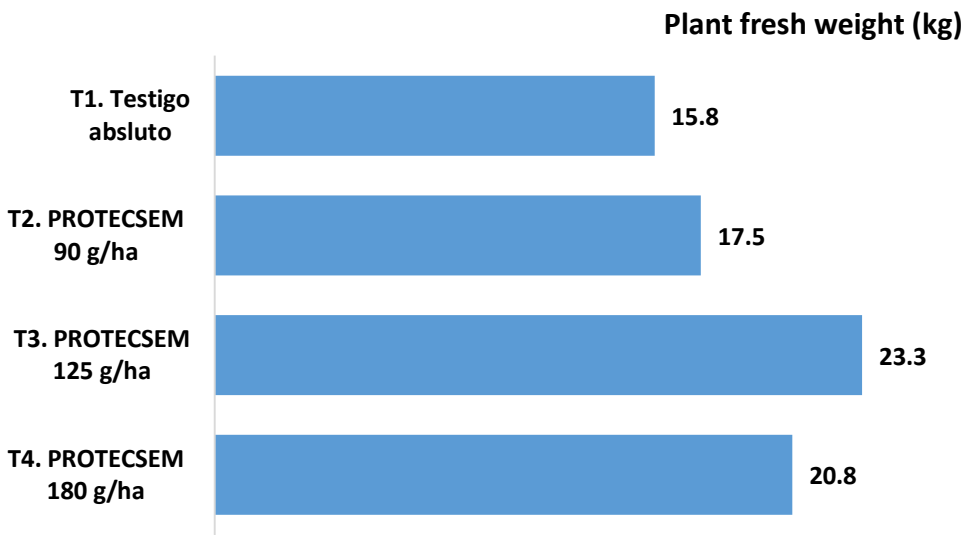

Figura 9. Fresh plant weight

10. Plant dry weight

In the analysis of variance performed with the dry plant weight data in the corn crop, significant differences were observed compared to the Untreated control and between treatments, at 60 days after planting and application. This was corroborated by a Tukey comparison of means (α=0.05).

It was shown that the dry plant weight was greater where PROTECSEM was applied at 125 g.ha^{-1}, with a mean of **4.4 kg**, and where it was applied at 180 g.ha^{-1}, and at 90 g.ha^{-1} with means of **3.7 kg** and **3.0 kg,** respectively, compared to the control that showed a mean of **2.3 kg** (Table 14)(Figure 10).

Table 14 . Evaluation of the plant **dry weight** variable (kg) in corn crop.

TREATMENTS	Plant dry wt
	60 dap
T1. Untreated control ("Testigo absoluto" in Figure)	2.3 B
T2. PROTECSEM (90 g.ha^{-1})	3.0 AB
T3. PROTECSEM (125 g.ha^{-1})	4.4 A
T4. PROTECSEM (180 g.ha^{-1})	3.7 A

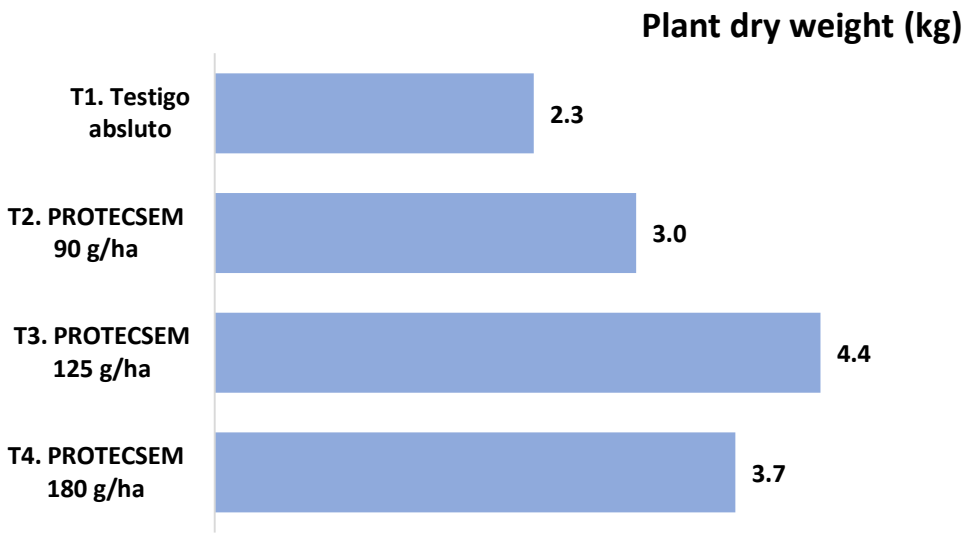

Figure 10 . Plant dry weight.

11. Fresh leafless ear weight

In the analysis of variance performed with the leafless ear weight data, significant differences were observed compared to the Untreated control and between treatments, at 160 days after planting. This was corroborated by a comparison of Tukey means (α=0.05).

It was observed that the ear fresh leafless ear weight was greater where PROTECSEM was applied at 180 g.ha$^{-1,}$ with a mean of **325 g,** and, although without significant differences, they were also numerically higher where it was applied at 125 g.ha^{-1} and 90 g.ha^{-1}, with means of **311 g** and **301 g**, respectively, compared to the control, that showed a mean of **288 g** (Table 15)(Figure 11)

Table 15 . Evaluation of ear fresh **weight without leaves,** in corn crop.

TREATMENTS	Ear fresh wt
	160 dap
T1. Untreated control (“Testigo absoluto” in Figure)	288.0 B
T2. PROTECSEM (90 g.ha^{-1})	301.0 AB
T3. PROTECSEM (125 g.ha^{-1})	311.0 AB
T4. PROTECSEM (180 g.ha^{-1})	325.0 A

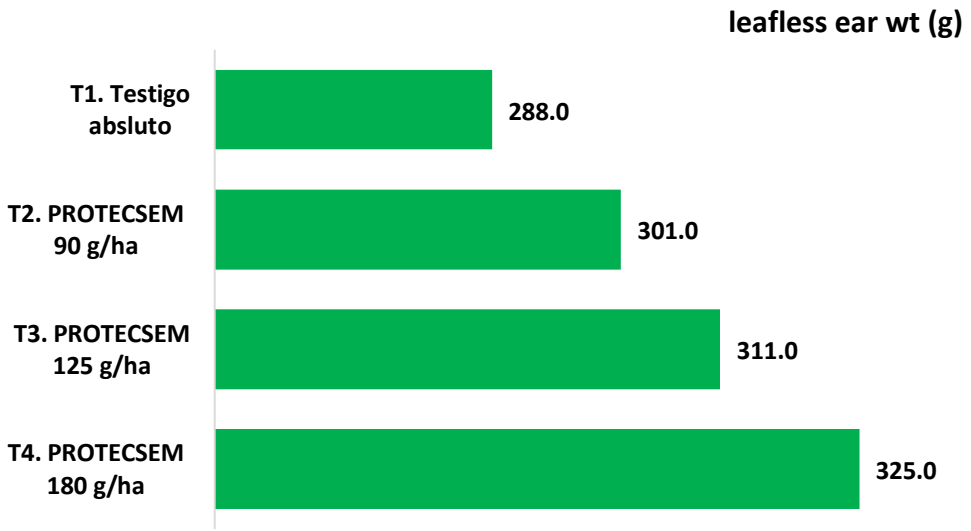

Figure 11 . Fresh leafless ear weight

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12. Number of ears per plant

The analysis of variance of the number of ears per plant data in corn crop showed no significant difference between treatments at 160 days after planting and application. This was corroborated by carrying out a Tukey comparison of means ($\alpha = 0.05$).

Table 16 . Evaluation of the variable **number of ears** in the corn crop.

TREATMENTS	Number ears/plant
	160 dap
T1. Untreated control("Testigo absoluto" in Figure)	1.0 A
T2. PROTECSEM (90 g.ha^{-1})	1.1 A
T3. PROTECSEM (125 g.ha^{-1})	1.0 A
T4. PROTECSEM (180 g.ha^{-1})	1.0 A

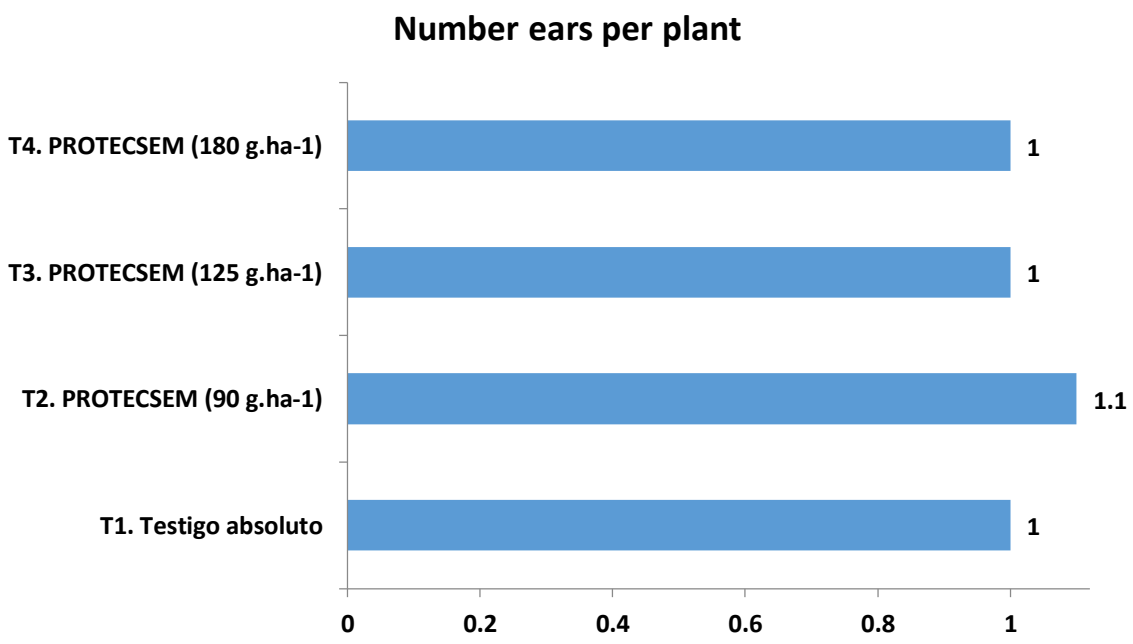


Figure 12. Number of ears per plant.

13. Corn Yield

In the analysis of variance carried out with the shelled corn yield data in the corn crop, significant differences were observed compared to the Untreated control, at harvest. This was corroborated by a Tukey comparison of means (α=0.05).

It was observed that the yield was higher where Protecsem was applied at 180 g.ha-^{1}, with a mean of **17 MT.ha^{-1}** (a 3.5 **MT.ha^{-1} or 26% increase above the control)** and although without significant differences, numerically differences were also shown where Protecsem was applied at 125 and 90 g.ha^{-1}, with **16.4** and **15.2 MT.ha^{-1}**, (**2.9 and 1.7 MT.ha^{-1} or 21 and 13% increases above the control),** respectively, compared to the control that showed a mean of **13.5 MT.ha^{-1}** (Table 17) (Figure 13).

Table 17. Evaluation of shelled corn yield (MT.ha^{-1} & bu/ac) in corn crop.

TREATMENTS	Shelled corn yield		Yield increase		
	bu/ac	MT.ha^{-1}	bu/ac	MT.ha^{-1}	%
T1. Untreated control	215 b	13.5 B		-	-
T2. Protecsem of Bio Seed (90 g.ha^{-1})	242 ab	15.2 AB	27	1.7	13
T3. Protecsem of Bio Seed (125 g.ha^{-1})	261 ab	16.4 AB	46	2.9	21
T4. Protecsem of Bio Seed (180 g.ha^{-1})	270 a	17.0 A	56	3.5	26

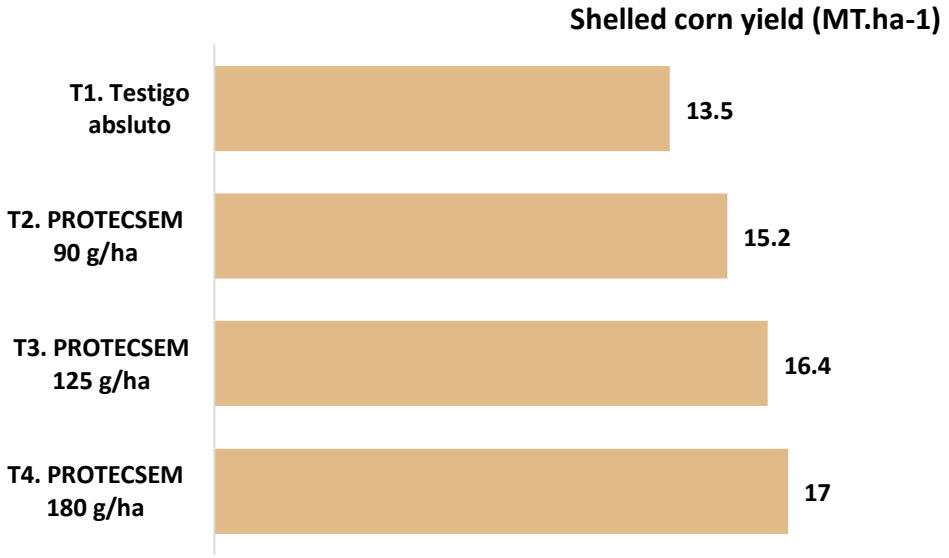


Figure 13 .Shelled Corn Yield.

PHYTOTOXICITY

The microbial inoculant PROTECSEM in its rates of 90, 125 and 180 $g.ha^{-1}$ was not phytotoxic in the corn crop.

CONCLUSIONS

The microbial inoculant PROTECSEM (BIO SEED in USA) in its rates of 90, 125 and 180 $g.ha^{-1}$, in seed treatment at planting, achieved positive effects on growth, greater vigor, increase in fresh and dry matter, and in general, in the yield of the corn crop, showing increases in the variables: dry and fresh root weight, root length, stem diameter, plant height, fresh and dry plant weight, fresh leafless ear weight and yield.

RECOMMENDATION

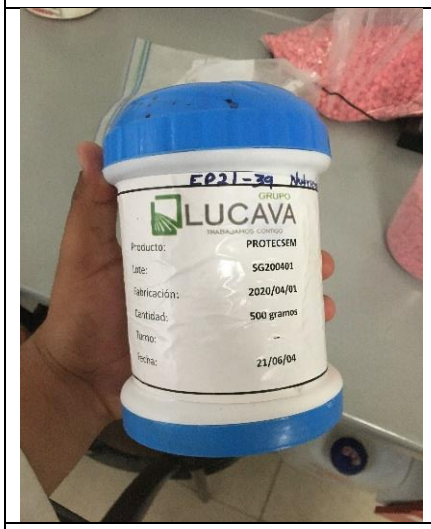
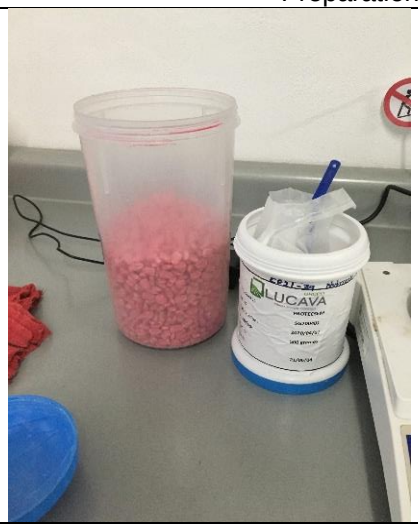
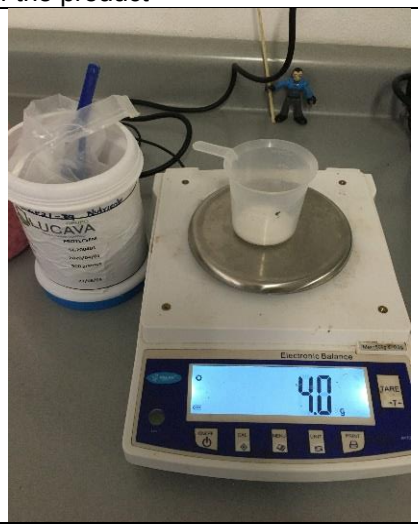
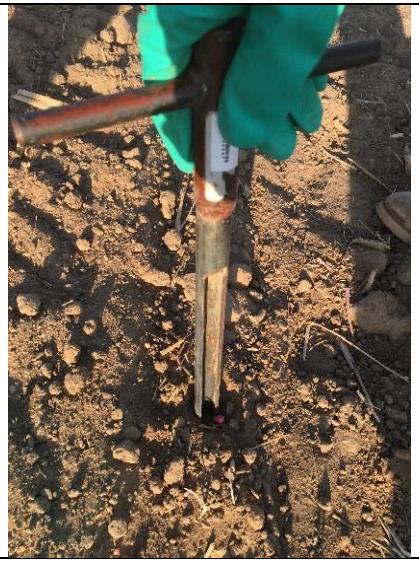
In summary, the recommendation for the microbial Inoculant PROTECSEM, is as follows:

Crop	Rate ($g \cdot ha^{-1}$)	Observations
Corn	90 to 180	Carry out an application in seed treatment, previously prepare a slurry at the rate of 7 ml of water per g of Protecsem and cover the seed well with it, let dry the seeds and sow according to the recommended crop practices.

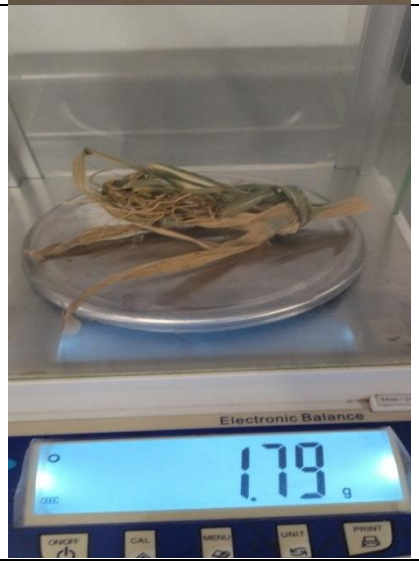
QUOTED LITERATURE

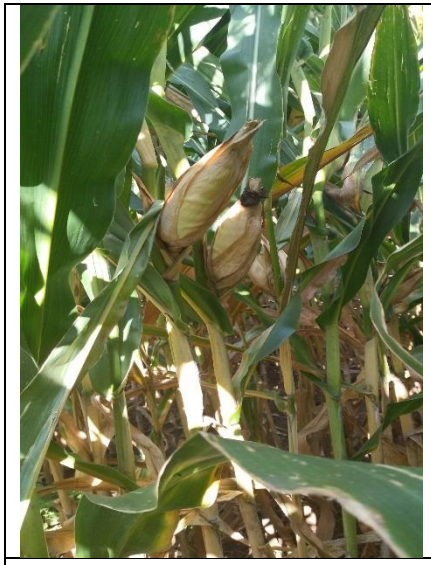
European Commission, 2009b. Regulation (EC) No 1107/ 2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/ EEC. Official Journal of the European Union L309: 1-50.

Muñetón P., P. 2009. La importancia de proteger al maíz como un bien común. Entrevista con la Dra. Elena Álvarez-Buylla Roces, jefa del departamento de Ecología Funcional, del Instituto de Ecología-UNAM. Revista Digital Universitaria. 10(4): 11.

Evaluation			
Preparation of the product			
			
Installation and planting			
			

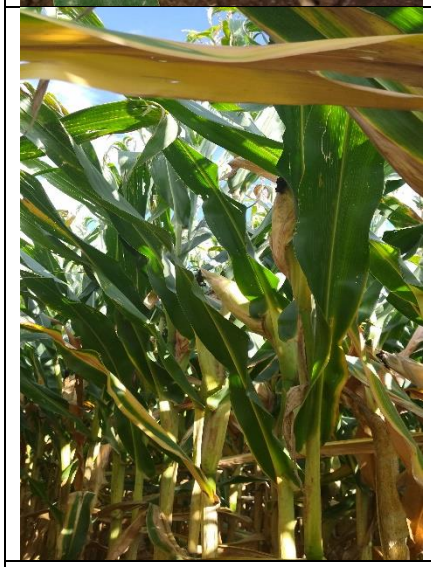
T1. Untreated control

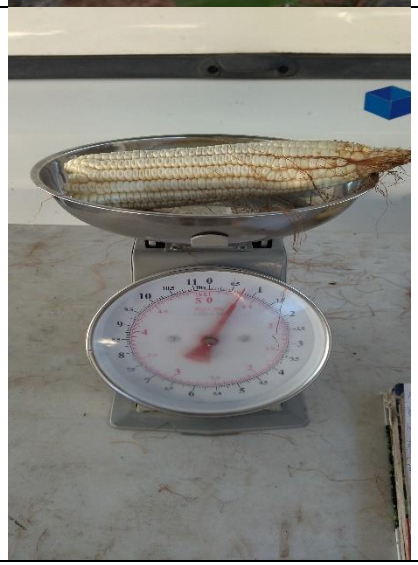





T2. PROTECSEM (90g / ha.)








T3. PROTECSEM (125g / ha.)

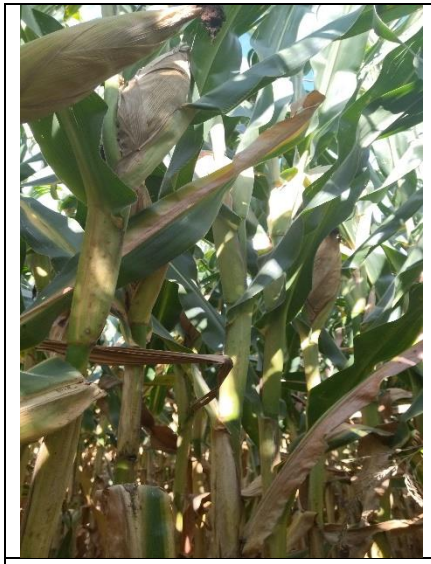

T3-R1

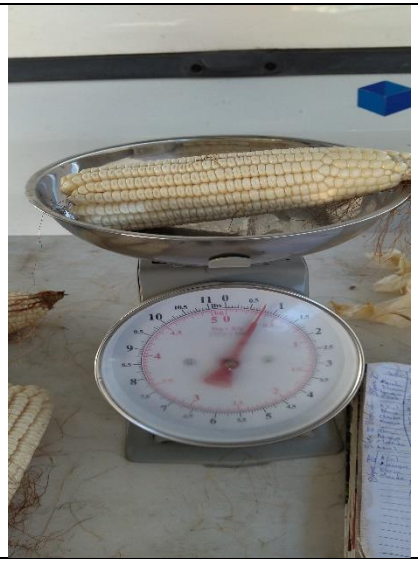



9.3


36.6
SPAD
ON
OFF
AVERAGE
ALL DATA CLEAR
DATA RECALL
1 DATA DELETE

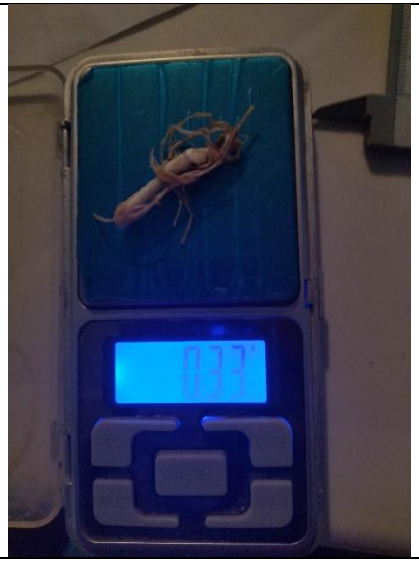
Electronic Balance
4.90 g

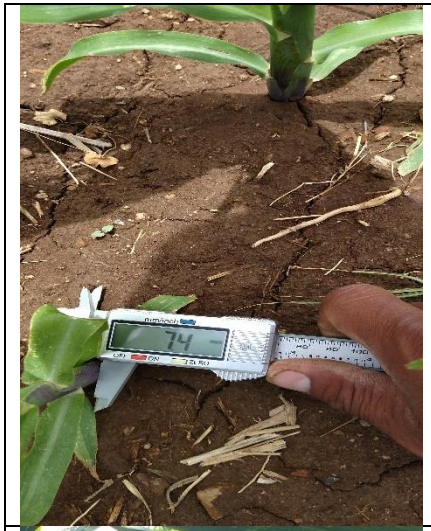





T4. PROTECSEM (180g / ha.)






7.4


43.7
SPAD
ON
OFF
AVERAGE
ALL DATA CLEAR
DATA RECALL
1 DATA DELETE

Electronic Balance
6.76 g
UNIT
PRINT