FINAL REPORT

EVALUATION OF BIOLOGICAL EFFECTIVENESS OF THE PRODUCT BRASINOFORTE (BIO SHOT IN USA), AS A GROWTH REGULATOR, IN CORN CROP, CARRIED OUT IN THE MUNICIPALITY OF GUASAVE, SINALOA.

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 .

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

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

c) Type of Input

()	Growth regulator	()	Organic or biological soil improver
(X)	Growth regulator	()	Inoculant
()	Moisturizer	()	Rooter
( )	Nutrient		

d) Report Title:

Evaluation of biological effectiveness of the product BRASINOFORTE, as a growth regulator, in corn crop, carried out in the municipality of Guasave, Sinaloa.

e) 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) Objectives:

1. Evaluate the biological effectiveness of the product BRASINOFORTE, as a growth regulator, in corn crop.

2. Determine the possible phytotoxic effects of the product BRASINOFORTE, as growth regulator in corn crop .

f) Commercial and / or experimental name.

- BRASINOFORTE

g) Guaranteed Composition:

Guaranteed composition	Concentration
Triacontanol	0.200%
Brassinosteroids	0.005%

MATERIALS AND METHODS

LOCATION OF THE EXPERIMENTAL SITE.

The study was installed in a commercial corn plot in the municipality of Guasave, Sinaloa.

h) Start date of the study: December 8, 2020

i) Study completion date: May 17, 2021

j) Crop in which the study was carried out :

Corn **Variety:** PIONNER P3260W

k) Phenological (growth) stage of the plant:

Sowing, vegetative and production stages

l) Trial layout

1. The trial was established under a completely randomized blocks layout, with four replicates.

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 320 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:

BLOCK I	BLOCK II	BLOCK III	BLOCK IV
T4	T1	T 2	T 5
T3	T2	T4	T 1
T1	T 5	T3	T4
T2	T4	T 5	T3
T5	T3	T1	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 BRASINOFORTE as growth regulator , in corn crop.

Tr .	**Product**	**Rate mL / ha**		**Rate mL / unit exp . (16 m^2)**		**Rate mL / Treatment (64 m^2)**	
		1st seed	2nd foliar	1st seed	2nd foliar	1st seed	2nd foliar
1	Absolute control						
2	Brasinoforte	25	100	0.04	0.16	0.16	0.64
3	Brasinoforte	25	150	0.04	0.24	0.16	0.96
4	Brasinoforte	25	200	0.04	0. 32	0.16	1.28
5	Vitazyme *	250	1000	0.40	1.60	1.60	6.40
2-5	Water	500 in tr . 2 - 4 and 250 in tr . 5	400,000	0.8 in tr . 2 - 4 and 0.4 in tr. 5	640	3.2 in tr . 2 - 4 and 1.6 in tr. 5	2560

* Commercial or regional control.

The first application was made in seed treatment. For treatments 2 to 4 (Brasinoforte), 25 mL /25 kg of seed was applied, using 500 mL of water; while for

treatment 5 (Vitazyme) 250 mL /25 kg of seed was applied , using 250 mL water of water.

Note: Taking into account that the weight of 60,000 seeds weighs 17.49 kg.

Planting density was 90 thousand plants/ ha.

The second application was carried out in a foliar way, 30 days after the first application.

o) Timing and number of applications

Two applications were carried out, with an interval of 30 days between each.

Application methods: The first application was made in treatment to the seed and the second application in spray to leaves (foliar).

Application equipment

1. **Seed treatment:** 5 L capacity jars.
2. **Foliar application:** A motorized sprayer with adjustable cone nozzle, was used.

Volume of water used

1. Seed treatment: 500 mL / seed 1 ha with Brasinoforte (Tr . 2 - 4) ; 250 mL / seed 1 ha with Vitazyme (Tr . 5)
2. Foliar application: 400 $L.ha^{-1}$

p) Other inputs used in the evaluation:

No other type of input which could interfere with the development of this trial, was used

r) Variables for estimating biological effectiveness and evaluation method.

1. Phytotoxicity . Assessed at 30 days after each application 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 BRASINOFORTE in growing corn.

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 percentage scale of EWRS to a percentage scale. ** Limit of acceptability.

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

3. Emergence in the field: at 14 days after planting, the emergence % per meter was measured.

4. Root fresh and dry weight: 3 plants were taken per experimental unit at 14 days after sowing and the fresh and dry weight of roots was taken.

5. Root length: 3 plants were taken per experimental unit 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 per experimental unit (plot), 30 days after the second application. The results are reported in mm.

7. Plant height (cm): was measured with a measuring tape in 3 plants at random per experimental unit, at 30 days after the second application. 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 30 days after the second application.

9. Fresh weight of the plant. 3 plants were taken per plot and weighted in a scale at 30 days after the second application.

10. Dry weight of the plant. . 3 plants were taken per plot and weighted in a scale at 30 days after the second application.

11. Fresh weight of the ear without leaves: 5 ears were taken per experimental unit and weighed.

12. Number of ears per plant: 5 plants were taken per experimental unit and the number of ears was counted .

13. Yield ($t.ha^{-1}$): The ears were harvested and weighed per linear meter per experimental unit and the yield was obtained .

s) Evaluation method, which should 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, 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.

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

u) CALENDAR OF ACTIVITIES. It is shown in Table 4 .

Table 4 . Calendar of activities of the evaluation study of biological effectiveness of the product BRASINOFORTE in the crop of corn .

ACTIVITY	**DATE**
1st application and seed planting	December 8 , 2020
Germination evaluations	December 15 and 22, 2020
2nd application (foliar) & phyto evaluation (30 da1a)	January 08, 2021
Growth & phyto variables evaluation (60 da1a)	February 07 , 2021
Harvest variable evaluation (160 da1a))	May 17, 2021

Da1a. days after the first application,

RESULTS AND DISCUSSION

1. Tray emergence or germination (%)

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

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

TREATMENTS	% of emergency
T1. Absolute control (Testigo absoluto in graph)	94.0 A
T2. Brasinoforte (25 mL seed & 100 mL foliar / ha) + water	93.8 A
T3. Brasinoforte (25 mL seed & 150 ml foliar / ha) + water	95.0 A
T4. Brasinoforte (25 mL seed & 200 mL foliar / ha) + water	93.3 A
T5. Vitazyme * (250 mL Seed & 1000 mL foliar / ha) + water	93.3 A

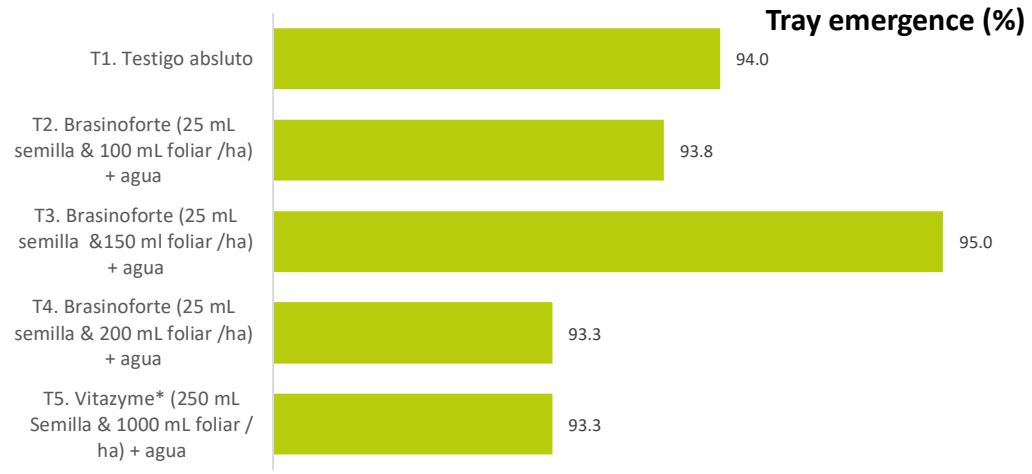

Figure 1 . Tray emergence

2. % Emergence or germination in the field

The analysis of variance performed of **emergence (germination) in field** data in growing corn showed no significant differences between treatments evaluated. This was corroborated by carrying out a comparison of means ($\alpha = 0.05$).However, It is worth mentioning that where growth regulator was applied, there was greater germination.

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

TREATMENTS	% emergence
T1. Absolute control (Testigo absoluto in graph)	83.3 A
T2. Brasinoforte (25 mL seed & 100 mL foliar / ha) + water	86.1 A
T3. Brasinoforte (25 mL seed & 150 ml foliar / ha) + water	91.7 A
T4. Brasinoforte (25 mL seed & 200 mL foliar / ha) + water	91.7 A
T5. Vitazyme * (250 mL Seed & 1000 mL foliar / ha) + water	88.9 A

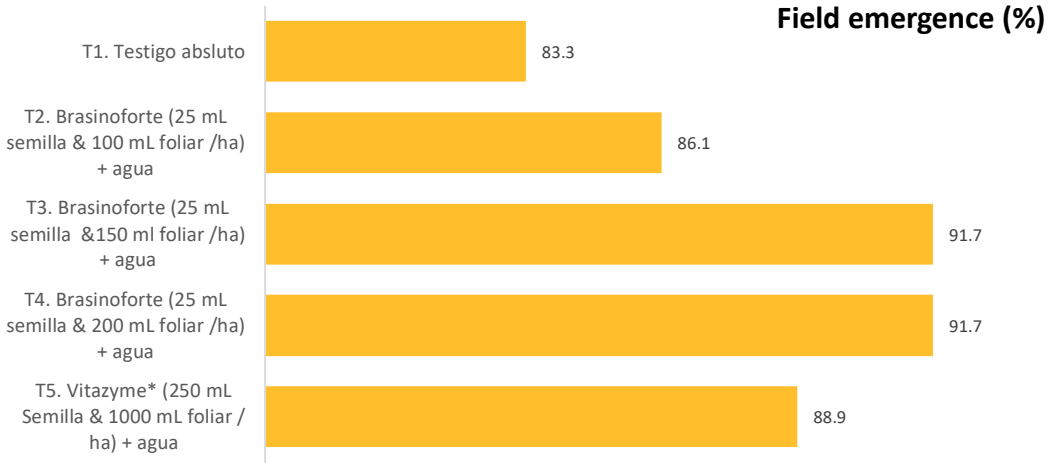

Figure 2 . Field emergence

3. Root fresh weight

The analysis of variance performed of the **root fresh weight** data in growing corn showed no significant difference between treatments. This was corroborated by carrying out a comparison of means ($\alpha = 0.05$). However, numerically and agronomically, a greater weight of the root was observed where Brasinoforte was applied.

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

TREATMENTS	PFR (g)
T1. Absolute control (Testigo absoluto in graph)	23.3 A
T2. Brasinoforte (25 mL seed & 100 mL foliar / ha) + water	25.8 A
T3. Brasinoforte (25 mL seed & 150 ml foliar / ha) + water	25.4 A
T4. Brasinoforte (25 mL seed & 200 mL foliar / ha) + water	26.3 A
T5. Vitazyme * (250 mL Seed & 1000 mL foliar / ha) + water	24.6 A

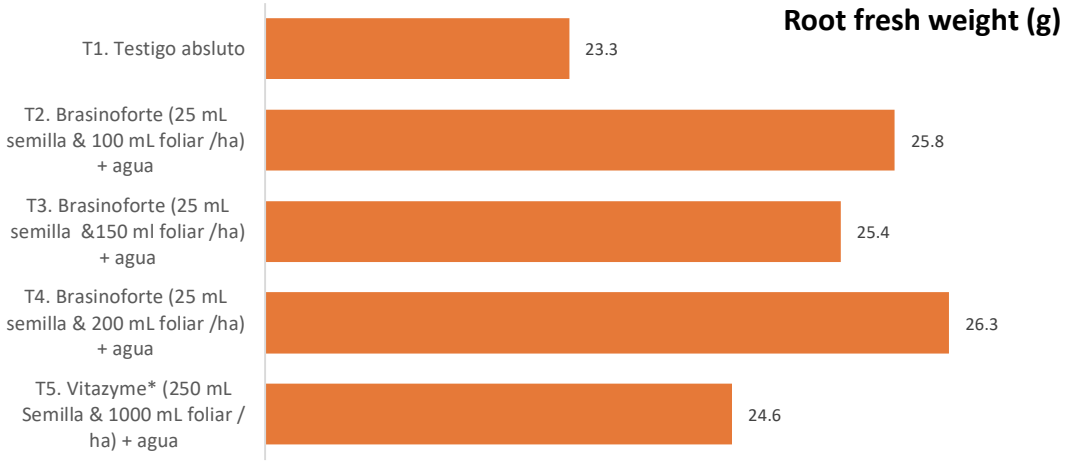

Figure 3 Root fresh weight

4. Root dry weight

The variance analysis performed of **root dry weight** data in corn crop showed significant differences between treatments. This was corroborated by carrying out a comparison of means (with $\alpha = 0.05$).

It was observed that the **dry weight of the root** was higher where Brasinoforte was applied at (25 mL seed & 200 mL foliar / ha) + water, showing an average of **16.8 grams** compared to the absolute control that showed an average **8.3 grams** (Table 8).

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

TREATMENTS	Root dry wt (g)
T1. Absolute control (Testigo absoluto in graph)	8.3 B
T2. Brasinoforte (25 mL seed & 100 mL foliar / ha) + water	15.3 AB
T3. Brasinoforte (25 mL seed & 150 ml foliar / ha) + water	14.8 AB
T4. Brasinoforte (25 mL seed & 200 mL foliar / ha) + water	16.8 A
T5. Vitazyme * (250 mL Seed & 1000 mL foliar / ha) + water	16.0 AB

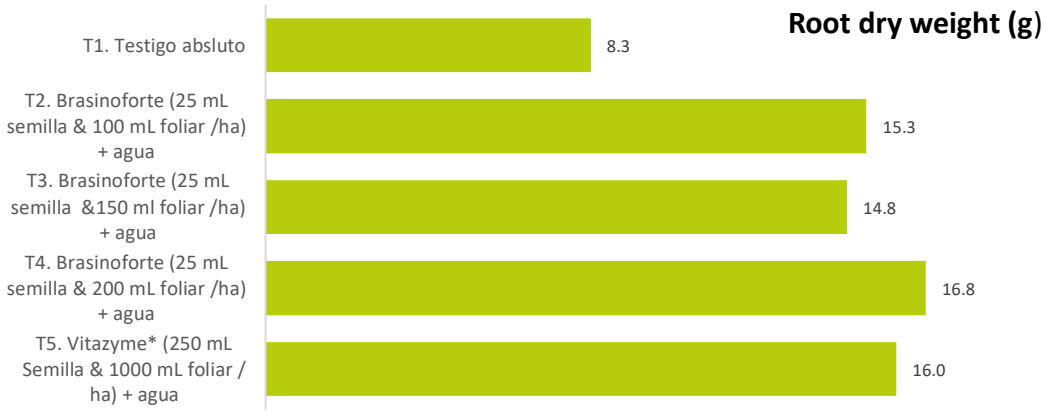

Figure 4 . Root dry weight .

5. Root length

The analysis of variance of **root length** data in corn crop showed significant differences between treatments. This was corroborated by carrying out a comparison of means (with $\alpha = 0.05$).

It was observed that the **root length** was greater where Vitazyme was applied at (250 mL Seed & 1000 mL foliar / ha) + water and Brasinoforte (25 mL seed & 200 mL foliar/ ha) + water, showing means of **18.1 cm and 17.2 cm** , respectively , in comparison with the absolute control that showed a mean of **14.4 cm** (Table 9).

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

TREATMENTS	Root length (cm)
T1. Absolute control (Testigo absoluto in graph)	14.4 C
T2. Brasinoforte (25 mL seed & 100 mL foliar / ha) + water	16.1 ABC
T3. Brasinoforte (25 mL seed & 150 ml foliar / ha) + water	15.7 BC
T4. Brasinoforte (25 mL seed & 200 mL foliar / ha) + water	17.2 AB
T5. Vitazyme * (250 mL Seed & 1000 mL foliar / ha) + water	18.1 A

Figure 5 . Root length.

6. Stem diameter

E l variance analysis performed of the **stem diameter** data in corn crop showed differences significant between treatments. This was corroborated by carrying out a comparison of means (with $\alpha = 0.05$).

It was observed that **stem diameter** was greater where Brasinoforte was applied at (25 mL seed & 200 mL foliar / ha) + water, showing an average of **24.6 mm,** compared to the absolute control that showed an average of **21.9 mm** (Table 10).

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

TREATMENTS	Stem diameter (mm)
T1. Absolute control (Testigo absoluto in graph)	21.9 B
T2. Brasinoforte (25 mL seed & 100 mL foliar / ha) + water	23.4 AB
T3. Brasinoforte (25 mL seed & 150 ml foliar / ha) + water	23.9 AB
T4. Brasinoforte (25 mL seed & 200 mL foliar / ha) + water	24.6 A
T5. Vitazyme * (250 mL Seed & 1000 mL foliar / ha) + water	23.8 AB

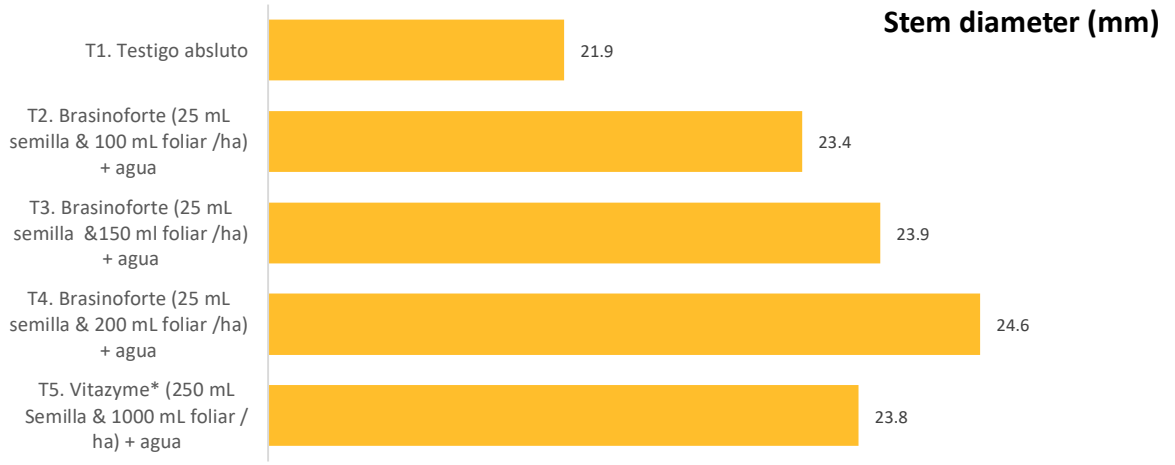

Figure 6 . Stem diameter.

7. Plant height

The analysis of variance performed of the **plant height** data in corn crop showed significant differences between the evaluated treatments. This was corroborated by carrying out a comparison of means (with $\alpha = 0.05$).

It was observed that **plant height** was higher where Brasinoforte was applied at (25 mL seed & 200 mL foliar / ha) + water and Vitazyme a (250 mL Seed & 1000 mL foliar / ha) + water, showing an average of **75.0 cm and 77.7 cm** respectively compared to the absolute control, that showed a mean of **58.2 cm** (Table 11).

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

TREATMENTS	Plant height (cm)
T1. Absolute control (Testigo absoluto in graph)	58.2 C
T2. Brasinoforte (25 mL seed & 100 mL foliar / ha) + water	66.7 B
T3. Brasinoforte (25 mL seed & 150 ml foliar / ha) + water	71.7 AB
T4. Brasinoforte (25 mL seed & 200 mL foliar / ha) + water	75.0 A
T5. Vitazyme * (250 mL Seed & 1000 mL foliar / ha) + water	77.7 A

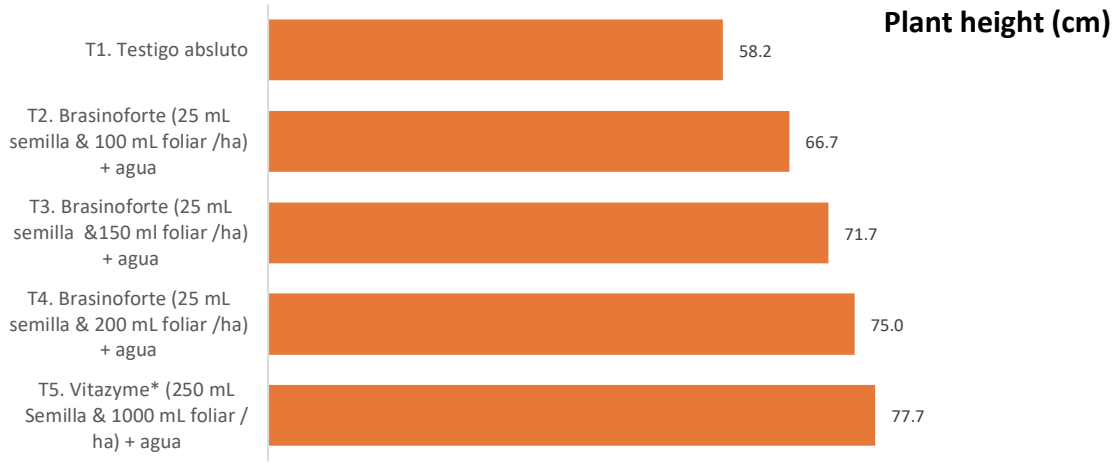

Figure 7 . Plant height .

8. Chlorophyll content

E l analysis of variance performed of the **chlorophyll content** data in growing corn showed significant differences between treatments. This was corroborated by carrying out a comparison of means (with $\alpha = 0.05$).

It was observed that the **chlorophyll content** was higher where Brasinoforte was applied at (25 mL seed & 200 mL foliar / ha) + water and (25 mL seed & 150 ml foliar / ha) + water, showing means of **49. 7 and 49. 9 SPAD units** respectively compared to the absolute control, that showed a mean of **45.8 SPAD units** (Table 12).

Table 12 . Evaluation of the variable **chlorophyll content** in the corn crop

TREATMENTS	SPAD
T1. Absolute control (Testigo absoluto in graph)	45.8 B
T2. Brasinoforte (25 mL seed & 100 mL foliar / ha) + water	48.8 AB
T3. Brasinoforte (25 mL seed & 150 ml foliar / ha) + water	49.9 A
T4. Brasinoforte (25 mL seed & 200 mL foliar / ha) + water	49.7 A
T5. Vitazyme * (250 mL Seed & 1000 mL foliar / ha) + water	48.7 AB

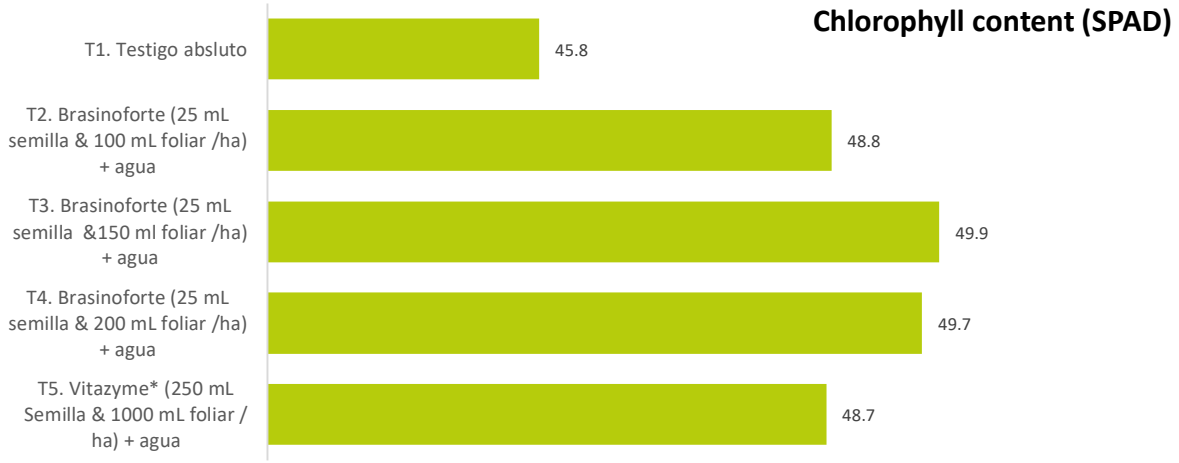

Figure 8. Chlorophyll content

9. Plant fresh weight

E l analysis of variance performed of the **plant fresh weight** data from in growing corn showed significant differences between treatments This was corroborated by carrying out a comparison of means (with $\alpha = 0.05$).

It was observed that the **plant fresh weight** was greater where Brasinoforte was applied at (25 mL seed & 200 mL foliar / ha) + water and Vitazyme at (250 mL Seed & 1000 mL foliar / ha) + water, showing means of **104.6 and 97.0 grams,** respectively, compared to the absolute control, that showed an average **74.6 grams** (Table 13).

Table 13 . Evaluation of p**lant fresh weight** variable in corn crop.

TREATMENTS	Plant fresh wt (g)
T1. Absolute control (Testigo absoluto in graph)	74.6 B
T2. Brasinoforte (25 mL seed & 100 mL foliar / ha) + water	85.0 AB
T3. Brasinoforte (25 mL seed & 150 ml foliar / ha) + water	91.7 AB
T4. Brasinoforte (25 mL seed & 200 mL foliar / ha) + water	104.6 A
T5. Vitazyme * (250 mL Seed & 1000 mL foliar / ha) + water	97.0 A

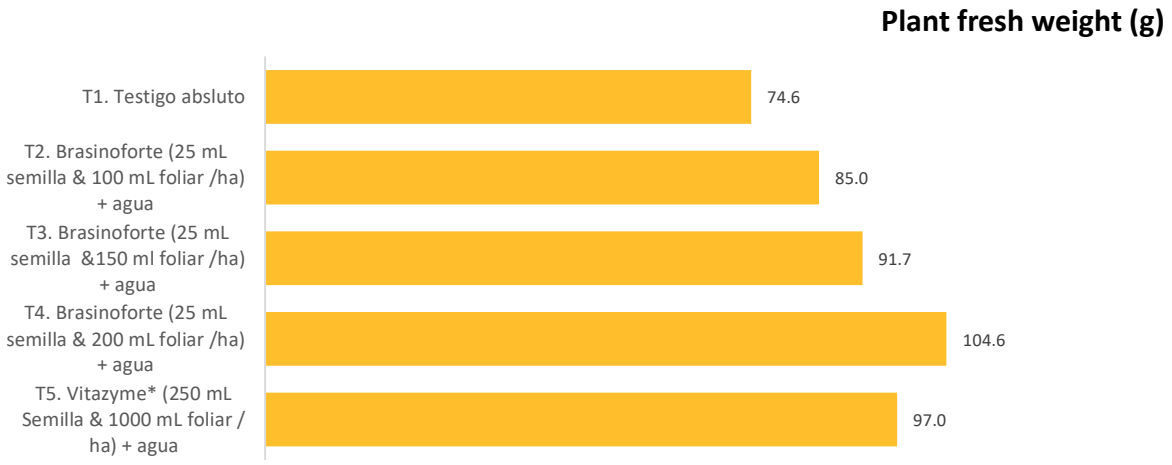

Figure 9 . Plant fresh weight

10. Plant dry weight

The analysis of variance performed of the **plant dry weight** data in corn crop showed significant differences between treatments applied. This was corroborated by carrying out a comparison of means (with $\alpha = 0.05$).

It was observed that the **plant dry weight** was greatest where Brasinoforte was applied at (25 mL seed & 200 mL foliar / ha) + water and Vitazyme at (250 mL Seed & 1000 mL foliar / ha) + water showing means of **76.3 and 72.5 grams** respectively compared to the absolute control, that showed an average **33.3 grams** (Table 14).

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

TREATMENTS	Plant dry wt (g)
T1. Absolute control (Testigo absoluto in graph)	33.3 B
T2. Brasinoforte (25 mL seed & 100 mL foliar / ha) + water	40.0 B
T3. Brasinoforte (25 mL seed & 150 ml foliar / ha) + water	44.0 B
T4. Brasinoforte (25 mL seed & 200 mL foliar / ha) + water	76.3 A
T5. Vitazyme * (250 mL Seed & 1000 mL foliar / ha) + water	72.5 A

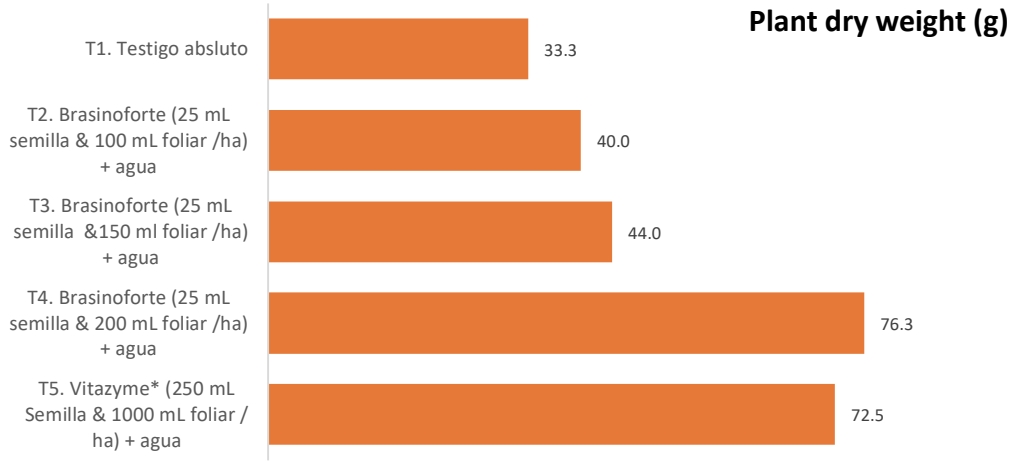

Figure 10 . Plant dry weight

11. Ear weight

The analysis of variance performed of the **ear weight** (without leaves) data in corn crop showed differences significant between treatments. This was corroborated by carrying out a comparison of means (with $\alpha = 0.05$).

It was observed that the **ear weight** was greater where Brasinoforte was applied at (25 mL seed & 200 mL foliar / ha) + water showing an average **492.0 grams,** compared to absolute control, which showed an average **421.8 grams** (Table 15).

Table 15 . Evaluation of the variable ear **weight** in corn crop.

TREATMENTS	Ear weight (g)
T1. Absolute control (Testigo absoluto in graph)	421.8 B
T2. Brasinoforte (25 mL seed & 100 mL foliar / ha) + water	472.3 AB
T3. Brasinoforte (25 mL seed & 150 ml foliar / ha) + water	447.3 AB
T4. Brasinoforte (25 mL seed & 200 mL foliar / ha) + water	492.0 A
T5. Vitazyme * (250 mL Seed & 1000 mL foliar / ha) + water	473.8 AB

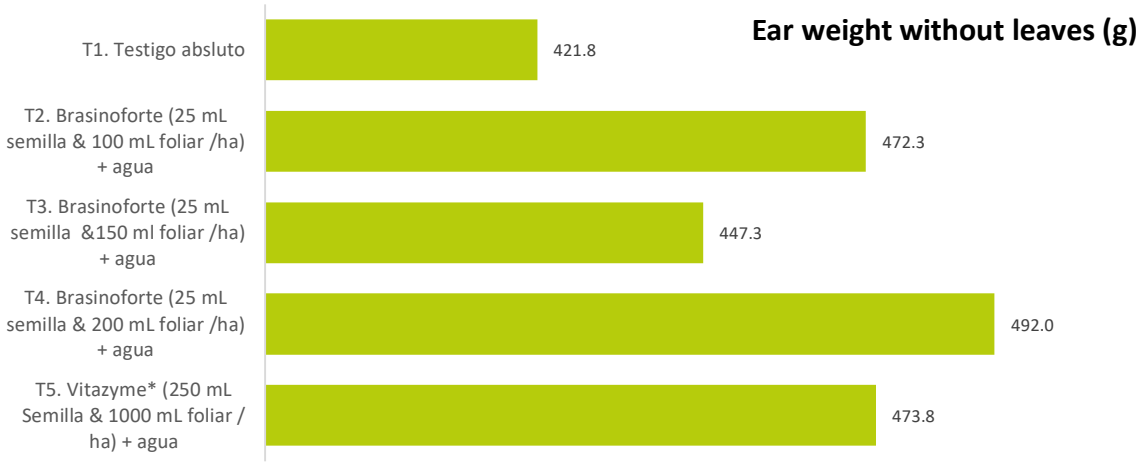

Figure 11 . Ear weight without leaves

12. Number of ears per plant

The analysis of variance performed of the **number of ears per plant** data in corn crop did not show significant differences between treatments. This was corroborated by carrying out a comparison of means (with $\alpha = 0.05$). However, numerically, a slightly larger number of ears was observed in all growth regulator treatments, compared to the absolute control.

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

TREATMENTS	Number ears/plant
T1. Absolute control (Testigo absoluto in graph)	2.3 A
T2. Brasinoforte (25 mL seed & 100 mL foliar / ha) + water	2.5 A
T3. Brasinoforte (25 mL seed & 150 ml foliar / ha) + water	2.6 A
T4. Brasinoforte (25 mL seed & 200 mL foliar / ha) + water	2.5 A
T5. Vitazyme * (250 mL Seed & 1000 mL foliar / ha) + water	2.5 A

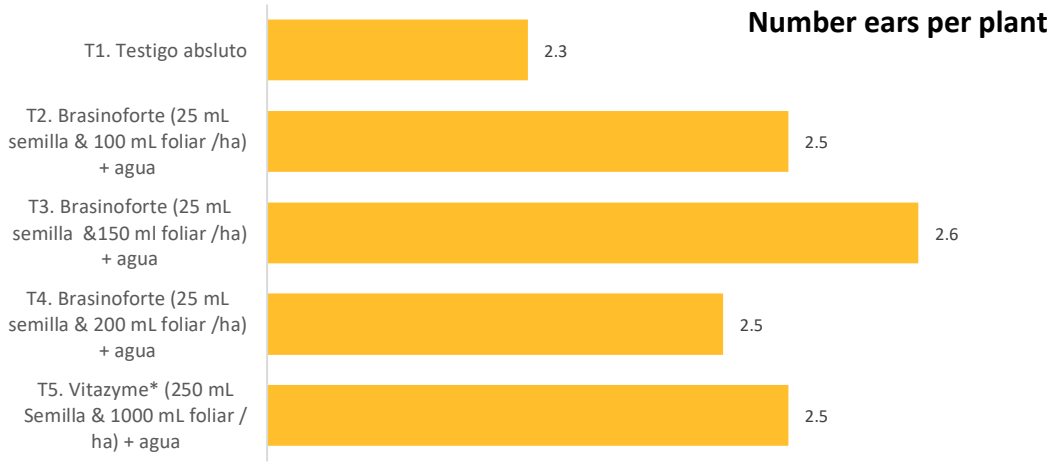

Figure 12 . Number of ears/plant

13. Corn Yield

The analysis of variance performed of the **yield** data **(MT.ha^{-1})** in corn crop did not show significant differences between treatments. This was corroborated by carrying out a comparison of means (with $\alpha = 0.05$) .

However, numerically and agronomically, a higher yield was observed where Brasinoforte was applied in its three different rates and in Vitazyme.

Table 17. Evaluation of the **yield** variable in corn crop

TREATMENTS	Yield		Yield increase		
	bu/ac	MT.ha^{-1}	bu/ac	MT.ha^{-1}	%
Absolute untreated control	138	8.7 A	138	-	-
Brasinoforte (25 mL seed & 100 mL foliar / ha) + water	170	10.7 A	170	2.0	23
Brasinoforte (25 mL seed & 150 ml foliar / ha) + water	167	10.5 A	167	1.8	21
Brasinoforte (25 mL seed & 200 mL foliar / ha) + water	172	10.8 A	172	2.1	24
Vitazyme * (250 mL seed & 1000 mL foliar / ha) + water	170	10.7 A	170	2.0	23

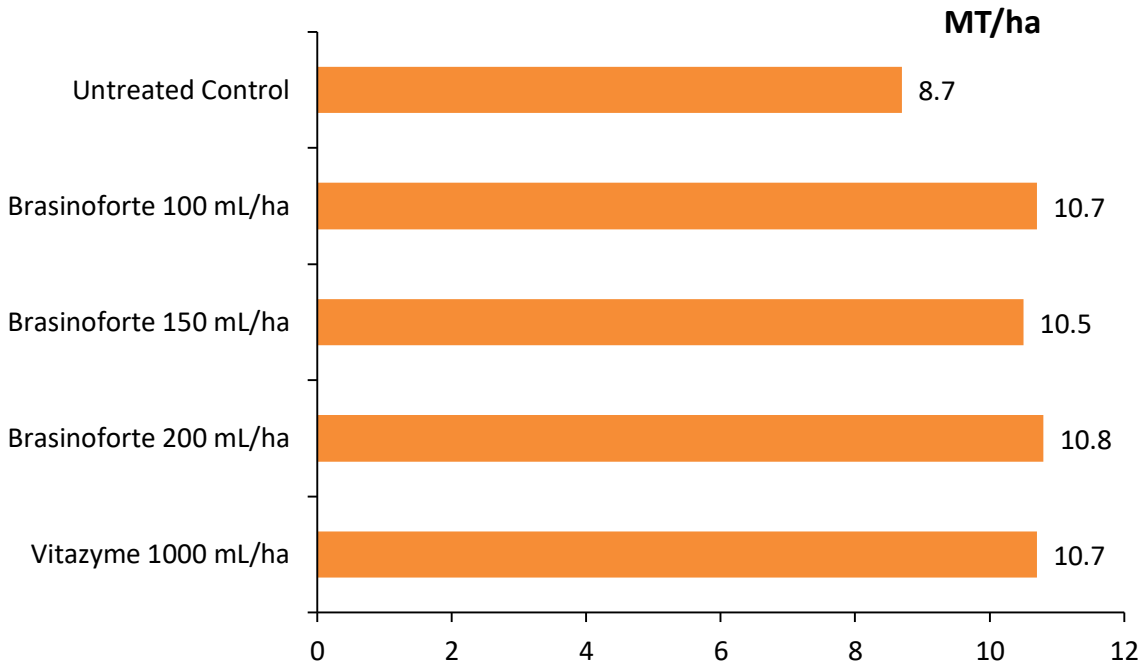

Figure 13 .Shelled Corn Yield.

PHYTOTOXICITY

The product Brasinoforte at rates of (25 mL seed & 100 mL foliar / ha) + water, (25 mL seed & 150 m L foliar / ha) + water, and (25 mL seed & 200 mL foliar / ha) + water and Vitazyme at (250 mL Seed & 1000 mL foliar / ha) + water, were not phytotoxic in growing corn .

CONCLUSIONS

The product Brasinoforte, in rates of (25 mL seed & 100 mL foliar / ha) + water, (25 mL seed & 150 ml foliar / ha) + water, and (25 mL seed & 200 mL foliar / ha) + water , achieved positive effects in planting, vegetative growth and production stages, by increased the variables; emerged plants ((germination), fresh and dry root weight, root length, stem diameter, plant height, chlorophyll content , plant fresh and dry weight, ear weight and number per plant and corn yield.

RECOMMENDATION

In summary, the recommendation for the product Brasinoforte is as follows:

Crop	Rate	Observations
Corn	25 mL / 25 kg seed treatment	Carry out the first application in seed treatment, using 500 mL of water. The second application is carried out in foliar spray at 30 days after the first application with a volume of water of 400 $L.ha^{-1}$.
	100 to 200 **$mL.ha^{-}$ (foliar application)**	