RESULTS OF THE FIELD TRIAL CARRIED OUT IN THE 2022 CAMPAIGN. TECHNICAL ASSISTANCE CONTRACT BETWEEN THE CENTER FOR SCIENTIFIC AND TECHNOLOGICAL RESEARCH OF EXTREMADURA (CICYTEX) AND THE COMPANY JOTRALOG TO CARRY OUT THE WORK:

"VERIFICATION OF AGRONOMIC EFFECTS OF PHYTO-C_3™ PRODUCTS ON AN OUTDOOR HORTICULTURAL CROP AS WELL AS ON THE IRRIGATION SYSTEM"

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1. INTRODUCTION

The report of results of the work carried out for the company JOTRALOG ESPAÑA S.L. is written. which has been developed at the Finca La Orden-Valdesequera Agricultural Research Institute (CICYTEX) under the contract signed on May 18, 2021 and extended until December 18, 2022, through the technical assistance "Verification of the agronomic effects of Phyto-C_3™ products in an outdoor horticultural crop, as well as as on the irrigation system."

2. OBJECTIVES

The objectives of the technical assistance have been:

- Objective 1: To examine the agronomic effects of two products, Phyto-C_3™ in an experimental plot on an outdoor crop of great interest to the region such as the tomato industry versus a control treatment, ifn application of product.
- Objective 2: To analyze the effects produced by Phyto-C_3™ on the irrigation system in industrial tomato.

3. TREATMENTS AND EXPERIMENTAL DESIGN

The test has been carried out in an experimental plot located in the Finca La Orden belonging to the Center for Scientific and Technological Research of Extremadura (CICYTEX) located in Las Vegas Bajas del Guadiana on an industrial tomato crop and different from the experimental plot of the trial conducted in 2021.

The cultivation techniques used were the usual ones in the area; the irrigation system was surface drip, and the plant material used was a medium-cycle industrial tomato variety, "H1015", transplanted on April 13, 2022 to a single row per horse, with a distance between plants of 25 cm and between beds of 1.50 m, with a planting density of 26,666 plants / ha. The harvest of the fruits was done between July 21, 2022, and 99 days after transplantation (ddt).

The total area of the trial was 576 m2 with experimental design of randomized blocks with 4 repetitions per treatment. Each experimental plot was composed of 4 beds of 8 meters in length separated by 1.5 m and on the two central rows all the samplings were carried out to avoid the "edge effect".

The tested treatments proposed and agreed with the company and were:

- T1: With application of Phyto-C_3™.
- T2: With application of organic Phyto-C_3™.
- T3: No application of Phyto-C_3™. It is the control treatment.

The distribution of treatments and blocks in the test plot is graphically described in the following image:

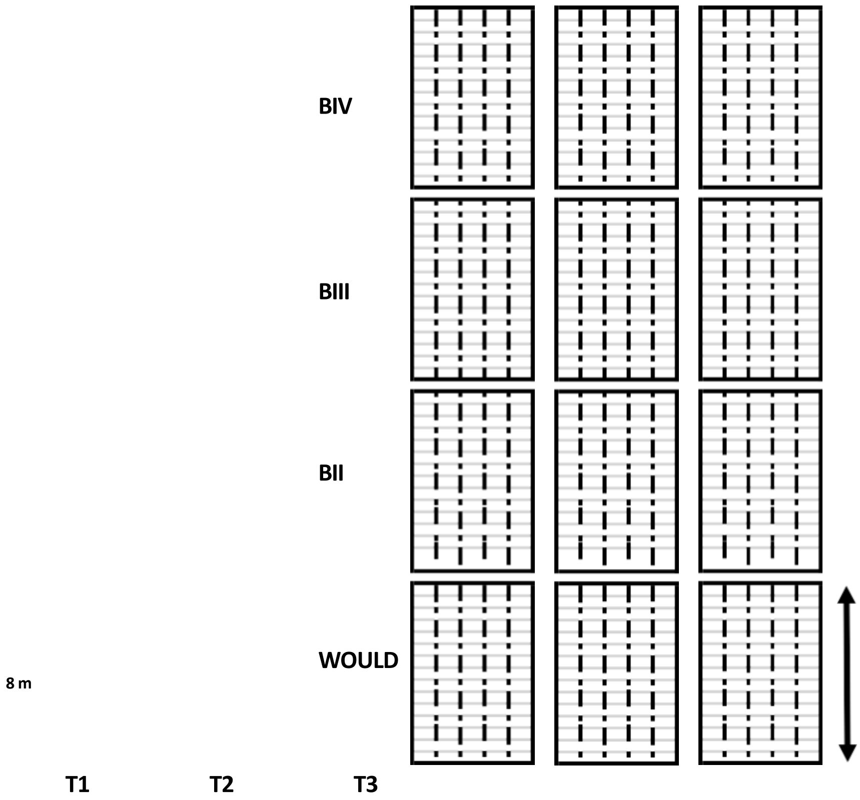

Figure 1. Distribution of the trial.

With regard to fertilization, all treatments were applied the same units of fertilizers, a total of 200 UF of nitrogen, 90 UF of phosphorus, 300 UF of potassium and 25 UF of calcium. The composition of the solutions was:

-Nitrogen solution: 20% N. 10% in nitric form and 10% in ammonia form.

-Phosphoric solution: 20% chloride-free $P2O_5$.

-Potassium solution: 15% K2O from potassium chloride.

The distribution of fertilizers is detailed in the table below:

Table 1. Distribution of fertilization throughout the vegetative cycle. "ddt": Days after transplantation.

Date implementation	of ddt	N (UF)	P (UF)	K (UF)	Like (UF)
25/04/2022	12	10	20	10	0
29/04/2022	16	10	20	10	0
06/05/2022	23	15	10	10	0
13/05/2022	30	15	10	10	0
23/05/2022	40	20	10	20	0
27/05/2022	44	20	4	20	0
02/06/2022	50	22	2	30	0
10/06/2022	58	22	2	30	0
16/06/200	64	22	2	40	10
23/06/2022	71	20	2	30	10
07/07/2022	85	20	4	60	5
13/07/2022	91	4	4	30	0
Total		200	90	300	25

The amount of water and product per treatment (T1 and T2) was 437.11 grams of Phyto Phyto-C_3™ and 87.02 m^3. The incorporation of Phyto-C_3™ into the treatments coincided with the application of fertilizers. The calculation for the application of Phyto-C_3™ products was made based on the volume of water applied since the previousfertilization, and always providing a concentration of 5 ppm in each of the applications.

To determine if there are statistical differences according to the type of product applied, the statistical test Anova of a Factor (Tukey) with a significance level $p<0.05$ and $p<0.01$ between treatments T1, T2 and T3 with the statistical program IBM Statistics 22 has been applied. In addition, in the results of the soil samples, a test has been applied for independent samples with a confidence interval of 95% and 99% to interpret the effect of the product on the soil samples after its application throughout the crop cycle in each of the T1 and T2 treatments.

4. PHYSICOCHEMICAL CHARACTERISTICS DTHE SOIL

A physicochemical characterization was performed in soil samples collected at a depth of 0 to 30 cm per treatment, at the beginning of the culture (prior to transplantation, 0 ddt) and after collection at 107 ddt . The interpretation of the results has been made based on the reference values published by González, M.C. (1990).

The results of the physicochemical characteristics at the beginning and end of the crop are shown in Tables 2 and 3 respectively, and indicate that the pH value is neutral at the beginning of cultivation in the three treatments, however, it is observed that at the end of culture it decreases for treatments T1 and T3 reaching acid pH values in T1. In all treatments the values of electrical conductivity (micros / cm) classify soil samples as non-saline, low in organic matter and C / N ratio and, high in P (ppm) and N (%). The K content (meq /100g) is low in T1 and T2 at the beginning of the crop, while in T3 it is high and after collection the T3 content is low compared to the initial sampling even becoming deficient. The concentration of Na (meq/100g) in the samples collected at 107 ddt is within normal with respect to the deficiencies presented by T1 and T2. The Ca content (meq/100g) in soil samples collected at 107 ddt is within normal, while at initial was deficiente for T1 and in excess for T3. The amount of Mg (meq/100g) in soil is high at the end of the crop while at the beginning it was low in T1 and high in T3.

Table 2. Physicochemical characteristics prior to transplantation in soil samples collected at a depth of 0-30 cm from treatments T1, T2 and T3. The reference values are those published by González, M.C. (1990).

Physicochemical characteristics	Home (0 ddt)			Reference values
	T1	T2	T3	
pH	7.1	6.7	7.5	6.6-7.5
EC (microS/cm)	79	72	85	0-2000
MOTotal (%)	0.8	0.8	0.7	2.0-2.5
P (ppm)	57	71	56	13-18
N (%)	0.25	0.30	0.25	0.11-0.20
C/N (%)	2	1	2	10-12
K (meq/100g)	0.28	0.48	0.80	0.51-0.75
Na (meq/100g)	0.2	0.3	0.6	0.6-1.0
Ca (meq/100g)	7	12	22	10-14
Mg (meq/100g)	1.4	2.2	4.2	1.6-2.5

Table 3. Physicochemical characteristics after collection (107 ddt) in soil samples collected at a depth of 0-30 cm from treatments T1, T2 and T3. The reference values are those published by González, M.C. (1990).

Physicochemical characteristics	Final (107 ddt)			Reference values
	T1	T2	T3	
pH	6.4	6.8	6.7	6.6-7.5
EC (microS/cm)	215	171	206	0-2000
MOTotal (%)	0.8	0.6	0.7	2.0-2.5
P (ppm)	34	21	74	13-18
N (%)	0.28	0.25	0.26	0.11-0.20
C/N (%)	2	1	2	10-12
K (meq/100g)	0.46	0.24	0.23	0.51-0.75
Na (meq/100g)	0.6	0.7	0.6	0.6-1.0
Ca (meq/100g)	11	13	12	10-14
Mg (meq/100g)	4.5	4.7	4.4	1.6-2.5

5. CONTENT OF N (N-NO_{3-} and N-NH_4^+) IN SOIL

Nitrogen content, measured in kg/ha as N-NO_{3-} and N-NH_4^+ was determined by spectrophotometry (Thermo Evolution 201 spectrophotometer) according to the Sempere et al. method. (1993) in the case of nitrates and as described by Rhine et al. (1998) in the case of ammoniums.

For its determination, soil samples were collected at a depth between 0 and 40 cm from each of the treatments, dried at room temperature, then screened with a sieve with a mesh light of 2 mm and over thefiltered samples were made the analyzes using for the extraction of nitrates, a solution of saturated calcium sulphate and for ammonium, potassium chloride 1M.

5.a. Pre-transplant N content (0 ddt)

The objective of this sampling before transplantation was to determine theinitial nitrogen content in both chemical forms since the first application of Phyto-C_3™ products was made 16 days later and the collection of samples was carried out prior to transplantation (0 ddt).

To the beginning of the cultivation the contents of N-NO_3^- (kg/ha) in the Treatments T1 y T2 sound similar while those of T3 are lower and with significant differences with T1 and T2 in this chemical form y in N total. (Figures 2, 3 y 4 y Picture 4). The concentration of N-NH_4^+ (kg/ha) it Similar in the three Treatments y without Differences Significant. In the Figure 4 herself Represents the Total content by N across of Its two chemical forms and Observed than in the Treatments T1 and T2 the contents of N-NO_3^- (kg/ha) y N-NH_4^+ (kg/ha) are in the same proportion, however, andn T3 a large part of the total N is in the form of N-NH_4^+ (kg/ha).

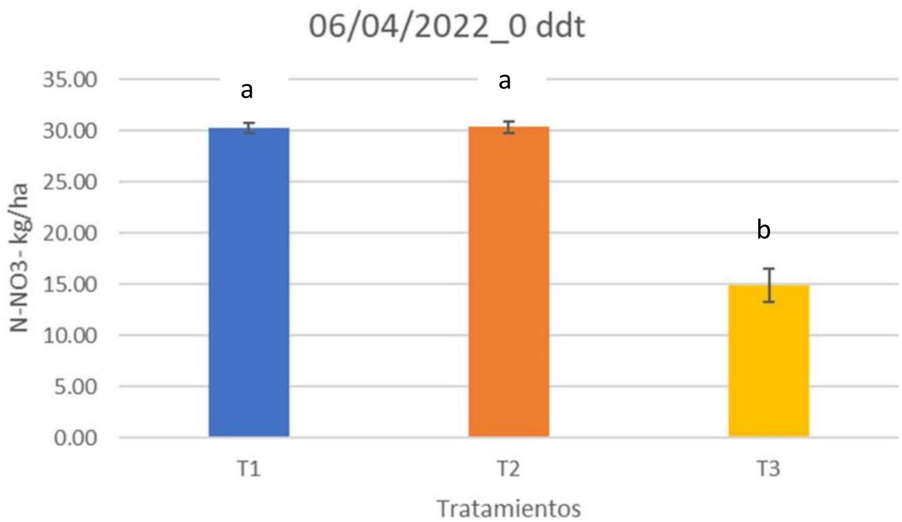

Figure 2. N-NO_3^- content (kg/ha) in the first 40 cm of the soil prior to transplantation. The average of the four repetitions and standard error of each treatment is represented. Different letters indicate significant differences, "*": Significant differences $p<0.05$ and "**": Significant differences $p<0.01$, according to the Tukey-b test.

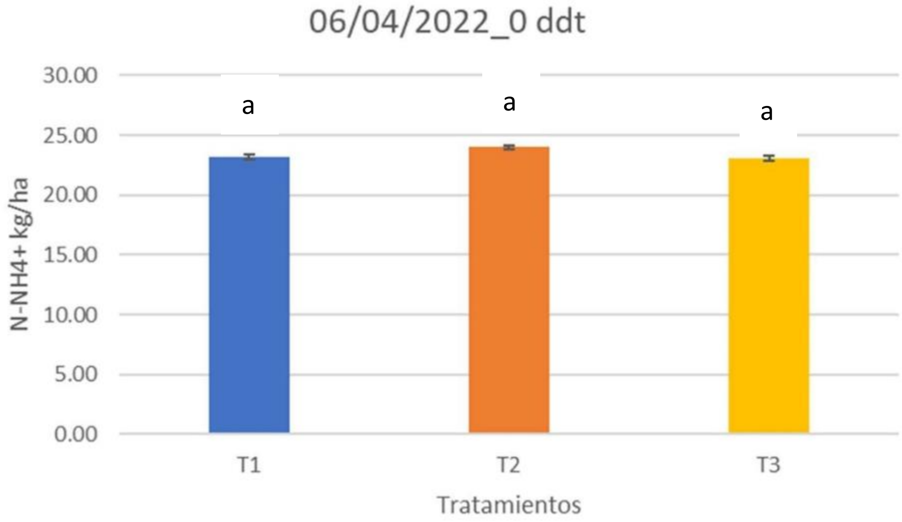

Figure 3. Content of N-NH $^{+}_{4}$(kg/ha) in the First 40 Cm of the soil prior to the transplantation. Herself It represents the average of the four repetitions and standard error of each treatment. Different letters indicate significant differences, "*": Significant differences p<0.05 and "**": Significant differences p<0.01, according to The test of Tukey-b.

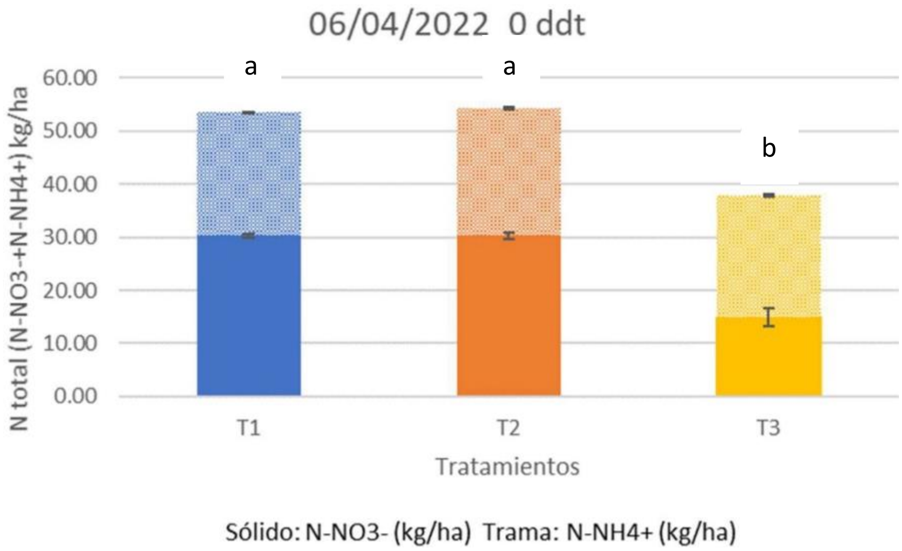

Figure 4. Total N content (N-NO$_3$- + N-NH 4$^+$) (kg/ha) in the first 40 cm of the soil prior to transplantation. The average of the four repetitions and standard error of each treatment is represented. Different letters indicate significant differences, "*": Significant differences p<0.05 and "**": Significant differences p<0.01, according to the Tukey-b test.

Table 4. N-NO$_3^-$ Content, N-NH$_4^+$ and Total N (N-NO$_3^-$ + N-NH$_4^+$) (kg/ha) in the first 40 cm of soil. The average of the four repeats and standard error of each pre-transplant treatment is represented. Different letters indicate significant differences, "*": Significant differences p<0.05 and "**": Significant differences p<0.01, according to The test of Tukey-b.

Treatments		N-NO3- (kg/ha)		N-NH4+ (kg/ha)		Total N (kg/ha)	
T1	Average	30.28	a	23.19	a	53.47	a
	EE	0.4599		0.2194		1.1552	
T2	Average	30.35	a	23.99	a	54.34	a
	EE	0.5705		0.1943		0.3821	
T3	Average	14.88	b	23.06	a	37.94	b
	EE	1.6271		0.2402		0.8964	
Signification		**		**		**	

5.b. N content after collection (107 ddt)

With sampling at 107 ddt The influence of the application of the products was analyzed Phyto-C$_3$™ American y Phyto-C$_3$™Organic respect of the control (without application of Phyto-C$_3$™) on the content of both chemical forms of N (NO$_3^-$ y NH$_4^+$) and total N in soil samples.

The concentration of N-NO$_3^-$ (kg/ha) No Sample Differences Significant between Treatments, however, a higher content is observed in the ARTT1 (Phyto-C$_3$™ American) (Figure 5 and Table 5). T1 treatment presents significant differences with T2 and T3 in N-NH$_4^+$ concentration (kg/ha) and with a higher content as for N-NO$_3^-$ (kg/ha) (Figure 6 and Table 5). Regarding the total N content (kg/ha) most of N is in the form of nitrates in all treatments, the T1 treatment has significant differences with the T3 treatment (Figure 7 and Table 5).

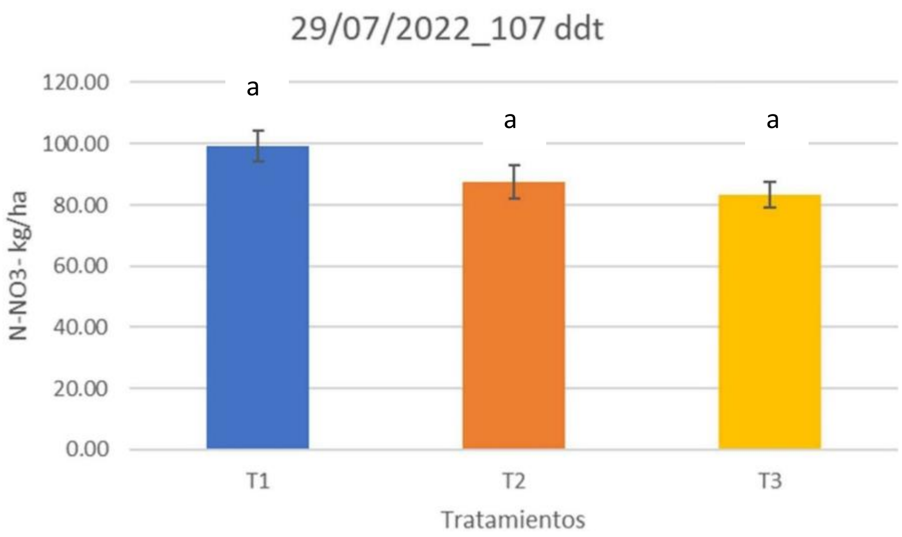

Figure 5. N-NO$_3^-$ content (kg/ha) in the first 40 cm of soil. The average of the four repeats and standard error of each treatment is represented at 107 ddt. Different letters indicate significant differences, "*": Significant differences p<0.05 and "**": Significant differences p<0.01, according to the Tukey-b test.

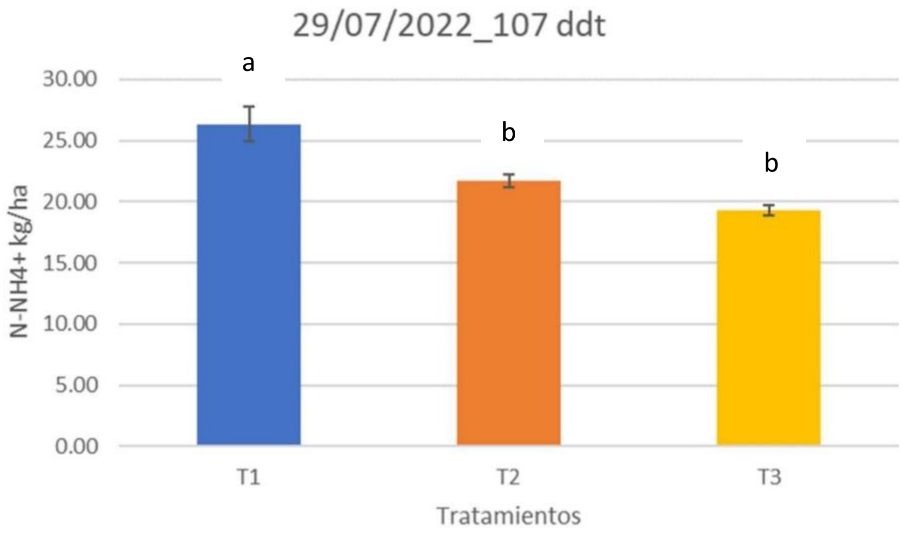

Figure 6. Content of N-NH + (kg/ha) in the First 40 Cm of the soil. The average of The four repetitions and standard error of each treatment at 107 ddt. Different letters indicate significant differences, "*": Significant differences p<0.05 and "**": Significant differencesas p<0.01, according to The test of Tukey-b.

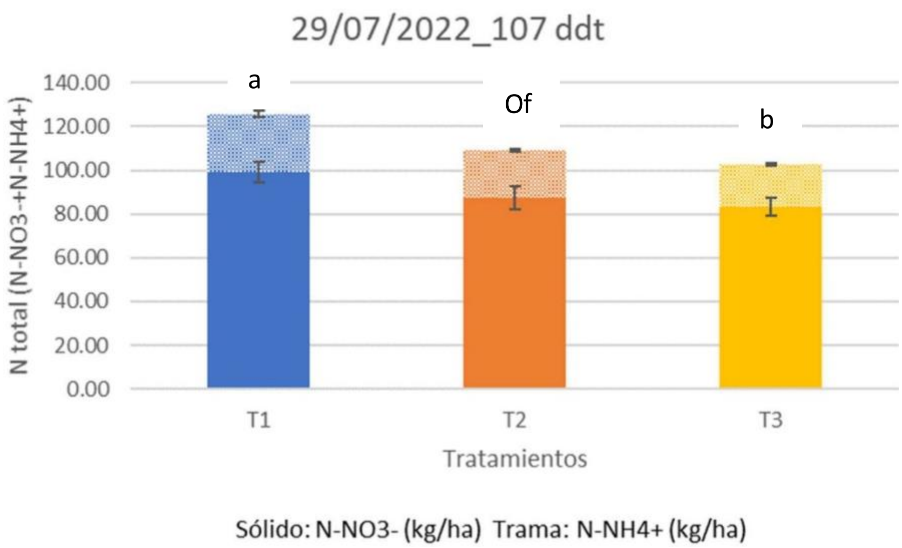

Figure 7. Total N content ($N\text{-}NO_3^-$ + N-NH 4$^+$) (kg/ha) in the first 40 cm of soil. The average of the four repeats and standard error of each treatment is represented at 107 ddt. Different letters indicate significant differences, "*": Significant differences p<0.05 and "**": Significant differences p<0.01, according to the Tukey-b test.

Picture 5. Content of N-NO$_3^-$, N-NH $_4^+$ y N total (N-NO $_3^-$ + N-NH $_4^+$) (kg/ha) in the First 40 Cm of the soil a the 107 ddt. Herself Represents the average of the four Repetitions y error standard of every treatment. Lyrics Different Indicate Differences Significant "*": Differences Significant p<0.05 y "**": Significant differences p<0.01, according to The test of Tukey-b.

Treatments		N-NO3- (kg/ha)		N-NH4+ (kg/ha)		Total N (kg/ha)	
T1	Average	99.25	a	26.35	a	125.60	a
	EE	4.8619		1.4183		3.7987	
T2	Average	87.35	a	21.71	b	109.07	Off
	EE	5.4423		0.5684		5.2067	
T3	Average	83.32	a	19.34	b	102.66	b
	EE	4.1660		0.4332		4.1413	
Signification		**		**		**	

5.c. Effect of treatments on N content

It has been compared by a test for independent samples with a confidence interval of 95 % and 99 % the effect of treatments with Phyto-C$_3$™ and control treatment on the content of N in soil samples collected at 0 ddt and 107 ddt .

The total soil N content at 107 ddt increases considerably compared to the soil sampling that was done prior to transplantation (0 ddt). Most of this N is in the form of nitrates with significant differencesin all treatments with a 99% confidence interval (Figure 8 and Table 6), and it is the T1 treatment that increases its concentration the most. On the contrary, ammonium contents do not increase in all treatments, only in T1 while in T2 and T3 they decrease, in all cases with significant differences to 99%. Although these results show that there are differences in the content of N (nitrates and ammoniums) between the initial and final sampling of soil, it has been previously seen that there are no differences between treatments in nitrate content to 107 ddt (Figure 5 and Table 5), but in ammonium (Figure 6 and Table 5).

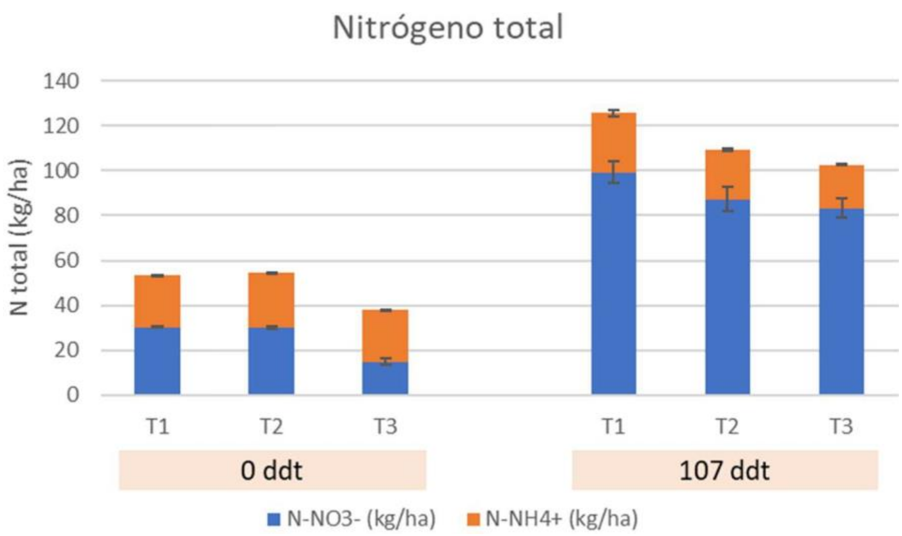

Figure 8. Content of N-NO 3-, N-NH 4+ and total N (N-NO 3- + N-NH 4$^+$) (kg/ha) in the first 40 cm of the soil. The average of the four repeats and standard error of each treatment at 0 and 107 ddt is represented.

Picture 6. Content of $N\text{-}NO_3^-$, $N\text{-}NH_4^+$ y N total ($N\text{-}NO_3^-$ + $N\text{-}NH_4^+$) (kg/ha) in the First 40 Cm of the soil at 0 and 107 ddt. The average of the four repetitions and standard error of each treatment is represented. "ns": Not significant, "*": Differences meansWith a 95% confidence interval and "**": Significant differences with a 99% confidence interval according to the T-test for samples Independent.

Treatments			N-NO3- (kg/ha)	N-NH4+ (kg/ha)	Total N (kg/ha)
T1	0 ddt	Average	30.28	23.19	53.47
		EE	0.4599	0.2194	1.1552
	107 ddt	Average	99.25	26.35	125.60
		EE	4.8619	1.4183	3.7987
Signification			**	**	**

Treatments			N-NO3- (kg/ha)	N-NH4+ (kg/ha)	Total N (kg/ha)
T2	0 ddt	Average	30.35	23.99	54.34
		EE	0.5705	0.1943	0.3821
	107 ddt	Average	87.35	21.71	109.07
		EE	5.4423	0.5684	5.2067
Signification			**	**	**

Treatments			N-NO3- (kg/ha)	N-NH4+ (kg/ha)	Total N (kg/ha)
T3	0 ddt	Average	28.41	22.48	50.89
		EE	0.4789	0.1783	0.9525
	107 ddt	Average	107.50	20.52	128.03
		EE	8.5140	0.1268	8.4529
Signification			**	**	**

Treatments			N-NO3- (kg/ha)	N-NH4+ (kg/ha)	Total N (kg/ha)
T4	0 ddt	Average	14.88	23.06	37.94
		EE	1.6271	0.2402	0.8964
	107 ddt	Average	83.32	19.34	102.66
		EE	4.1660	0.4332	4.1413
Signification			**	**	**

6. CONTENT OF $N\text{-}NO_3^-$ y $N\text{-}NH_4^+$ IN WATER OF IRRIGATION

The doses of irrigation water to the crop were adjusted according to the reference evapotranspiration and the Kc parameter of the crop, applying a total of 4532 m^3/ha. The concentration of nitrates and ammonium was determined by spectrophotometry with the Thermo Evolution 201 equipment following the methodology described by Sempere et al. (1993) in the case of nitrates and for ammoniums, by Rhine et al. (1998) in irrigation water samples that were collected from 22 to 98 ddt. The results are shown in Figure 9. Observe that the concentration of nitrates and ammonium present in the irrigation water presents a great variability and is different according to the moment of the analysis.

The total N content applied to the crop was 9.59 kg/ha and although it is a small part of the crop's needs it is important to consider this extra source of nitrogen.

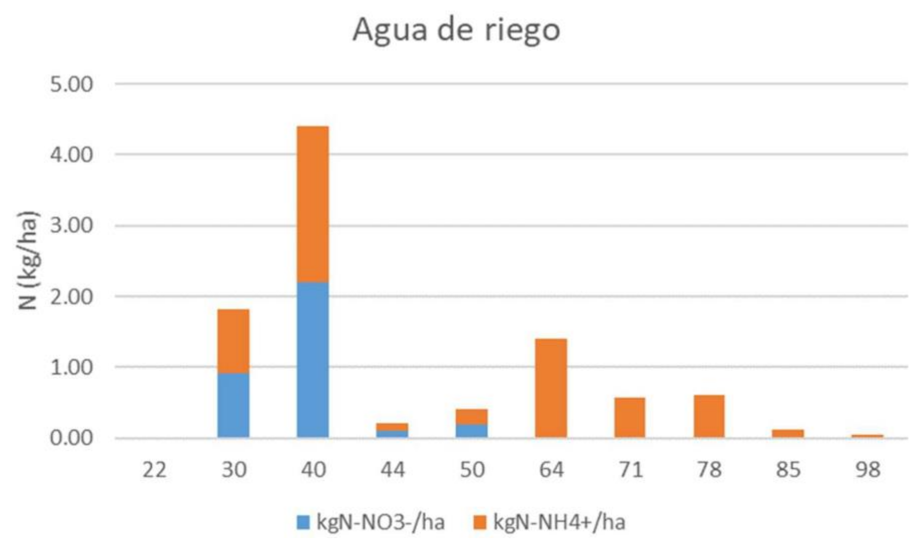

Figure 9. Content of $N\text{-}NO_3^-$ and $N\text{-}H_4^+$ in kg/ha in samples of water of irrigation to the long of the Development of the crop cycle.

7. CHARACTERIZATION OF THE NUTRITIONAL STATUS OF THE CROP

For the determination of the nutritional status has been used on the one hand non-destructive methods that allow a continuous monitoring of the crop and, characterized by its precocity in obtaining results and its ability to intervene on the cultivo as the reflectance meter on cover Crop Circle ACS 470, meters on sheet as SPAD Minolta 502 and Dualex Scientific Force A, compared to the classic method carried out through foliar analysis that leads to the destruction of the sample and slowness in results by the complex analytical processing.

7.a. Normalized vegetation index (NDVI and NDVIG)

With the Crop Circle ACS 470 (Hollands Scientifics) equipment, the most commonly used vegetation indices for the determination of crop status, the NDVI (Normalized Difference Vegetation Index) and NDVIG (Normalized Difference Vegetation Index) were determined. Green) from reflectance values at three wavelengths, 550, 670 and 760 nm on the vegetation cover. The evolution of both indices from 22 to 76 days after transplantation (ddt) is represented. It is a measure that integrates a growing area.

The stars in Figures 10 and 11 indicate significant differences between treatments and are detailed in Tables 7 and 8. It is observed that in all the phenological states measured there aresignificant differences. In the first sampling carried out at 22 ddt the treatment with the highest NDVI and NDVIG index is T3 (control treatment) and with differences significant with the rest of the treatments, however from this date it is T1 that presents a higher NDVI index and with significant differences, except for the 49 ddt that exceeds T2. The results of the NDVIG index are similar to the index antegerio, except that the T2 treatment has a higher value at both 49 and 63 ddt and that T1 at 76 ddt does not present significant differences with T2.

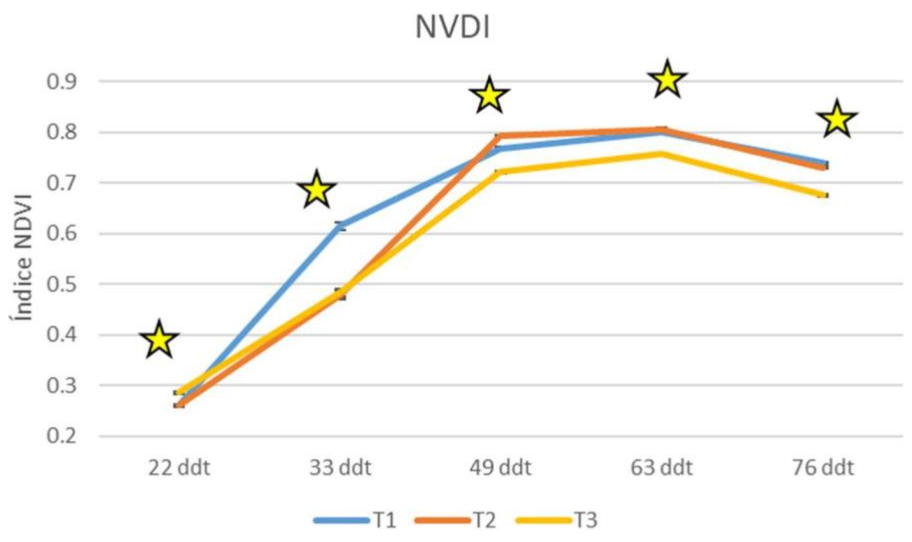

Figure 10. Evolution of the NDVI index throughout the crop cycle in the differenttreatments. Each point is the average of the four repetitions per treatment and its standard error. The stars indicate significant differences between treatments.

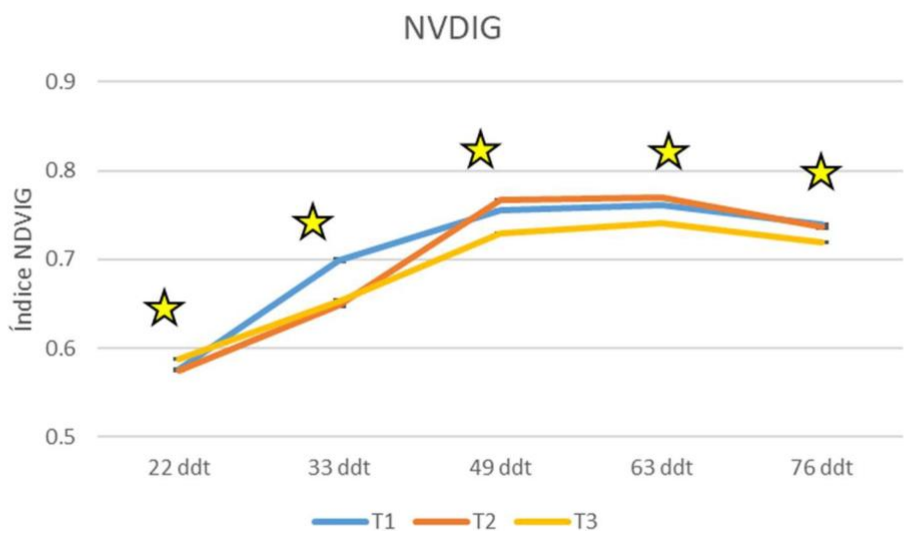

Figure 11. Evolution of the NDVIG index throughout the crop cycle in the different treatments. Each point is the average of the four repetitions per treatment and its standard error. The stars indicate significant differences between treatments.

The values of the NDVI index range from 0.261 to 0.806 compared to the NDVIG index that ranges from 0.577 to 0.767, therefore in this work, the NDVI index shows greater sensitivity against the NDVIG.

Table 7. Evolution of the NDVI index throughout the culture cycle in T1, T2 and T3 treatments. The average of the four repetitions per treatment and their standard error (EE) are indicated. Different letters in the same column imply significant differences, "*": Significant differences $p<0.05$ and "**": Significant differences $p<0.01$, according to the Tukey-b test.

NDVI											
Treatments		22 ddt		33 ddt		49 ddt		63 ddt		76 ddt	
T1	Average	0.261	b	0.615	a	0.768	b	0.801	a	0.739	a
	EE	0.0018		0.0074		0.0028		0.0021		0.0023	
T2	Average	0.260	b	0.479	b	0.793	a	0.806	a	0.730	b
	EE	0.0021		0.0086		0.0021		0.0023		0.0021	
T3	Average	0.286	a	0.483	b	0.720	c	0.756	b	0.675	c
	EE	0.0024		0.0071		0.0029		0.0018		0.0026	
Signification		**		**		**		**		*	

Table 8. Evolution of the NDVIG index throughout the crop cycle in treatments T1, T2 and T3. The average of the four repetitions per treatment and their standard error are indicated. Different letters in the same column imply significant differences, "*": Significant differences $p<0.05$ and "**": Significant differences $p<0.01$, according to the Tukey-b test.

NDVIG											
Treatments		22 ddt		33 ddt		49 ddt		63 ddt		76 ddt	
T1	Average	0.577	b	0.699	a	0.755	b	0.761	b	0.739	a
	EE	0.0007		0.0024		0.0012		0.0010		0.0010	
T2	Average	0.575	b	0.649	b	0.767	a	0.769	a	0.736	a
	EE	0.0008		0.0030		0.0010		0.0011		0.0010	
T3	Average	0.587	a	0.652	b	0.729	c	0.741	c	0.719	b
	EE	0.0008		0.0024		0.0011		0.0008		0.0010	
Signification		**		**		**		**		**	

7.b. Chlorophyll content (Minolta SPAD 502)

The concentration of chlorophyll present in the leaf was determined with the SPAD Minolta 502 equipment as an indicator of the nutritional status of the crop due to its close relationship with nitrogen content. 30 leaves per block and treatment were measured at 22, 33, 63 and 76 ddt, the same phenological states measured with the Crop Circle ACS 470.

Figure 12 and Table 9 show the results of the SPAD index and significance throughout the vegetative cycle. There are only significant differences between control treatment (T3) with T1 and T2 at 33 and 76 ddt. The control treatment had a lower SPAD index than the treatments with Phyto-C_3™ from 33 ddt and between the treatments with Phyto-C_3™ there are no significant differences.

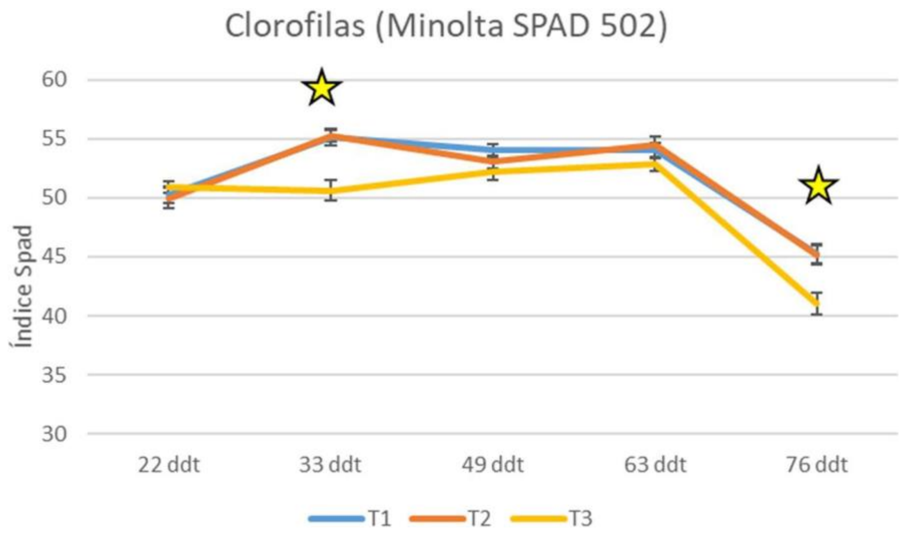

Figure 12. Evolution of the SPAD index throughout the crop cycle in the different treatments. Each point is the average of the four repetitions per treatment and its standard error. The stars indicate significant differences between treatments.

Table 9. Evolution of the SPAD index throughout the crop cycle in treatments T1, T2 and T3. The average of the four repetitions per treatment and their standard error are indicated. Different letters in the same column imply significant differences, "*": Significant differences p<0.05 and "**": Significant differences p<0.01, according to the Tukey-b test.

SPAD Index											
Treatments		22 ddt		33 ddt		49 ddt		63 ddt		76 ddt	
T1	Average	50.2	a	55.1	a	54.0	a	54.1	a	45.3	a
	EE	0.6978		0.7330		0.4970		0.5919		0.7736	
T2	Average	50.0	a	55.2	a	53.0	a	54.5	a	45.2	a
	EE	0.8394		0.5180		0.5220		0.6628		0.8267	
T3	Average	50.9	a	50.6	b	52.2	a	52.8	a	41.0	b
	EE	0.5278		0.8726		0.7959		0.5724		0.9085	
Signification		**		**		**		**		**	

7.c. Content of chlorophylls, flavonoids and anthocyanins

The Dualex® Scientific ForceA notebook (Orsay, France) provides an estimate of chlorophyll, flavonoid and anthocyanin levels of the leaf epidermis , without needing a previous preparation of the sample. This equipment provides an NBI index defined as the relationship between chlorophyll and flavonoid content. In this case it is a punctual measurement in leaves, making 30 measurements per elementary plot on each day of measurement that coincided with those made with Crop Circle and SPAD Minolta 502.

The stars indicate significant differencesbetween treatments. In chlorophylls (Figure 13 and Table 10) there are significant differences in control treatment (T3) with treatments with Phyto-C_3™ at 49 ddt and, in addition, at 76 ddt with organic Phyto-C_3™ (T2). The lowest value of this index throughout the cycle was for T3 except for 22 ddt that presented T1, on the contrary, the highest was observed in T2 (organic Phyto-C_3™).

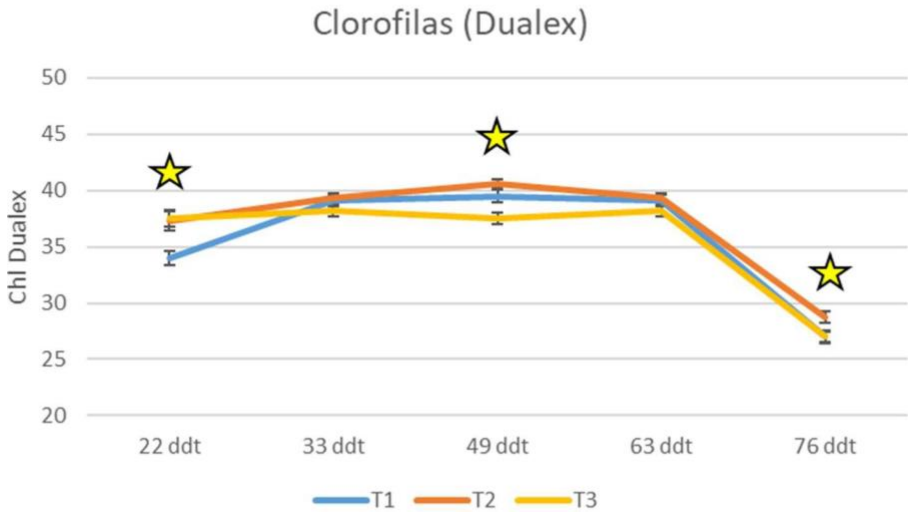

Figure 13. Evolution of the chlorophyll index measured with Dualex throughout the crop cycle in the different treatments. Each point is the average of the four repetitions per treatment and its standard error. The stars indicate significant differences between treatments.

The evolution of flavonoids throughout the vegetative cycle (Figure 14 and Table 10) indicates that at 49 ddt there were no significant differences between treatments, the T3 treatment pres entered the highest values and with significant differences with T1 and also with T2 at 33 and 63 ddt. The treatment with the lowest index was represented by T1. Flavonoids are protective agents in plants, therefore, a high content indicates that the plant is protecting itself from certain factors that affect optimal cellular functioning.

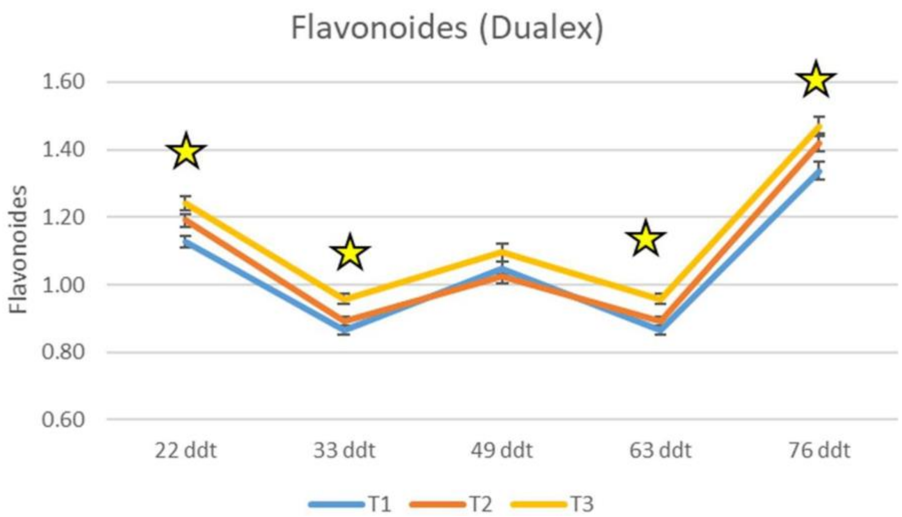

Figure 14. Evolution of the flavonoid index measured with Dualex throughout the culture cycle in the different treatments. Each point is the average of the four repetitions per treatment and its standard error. Stars indicate significant differencesbetween treatments.

Anthocyanins as well as flavonoids are pigments whose function is to protect the plant, therefore, an increase in these compounds are indicators that the plant is subject to some type of stress that altersits functioning. The T3 treatment had the highest index throughout the vegetative cycle and with significant differences at 49 ddt with T1 and T2 and at 76 with T1 (Figure 15 and Table 10). On the opposite side is the T1 treatment that presented the lowest index of all the treatments in the trial.

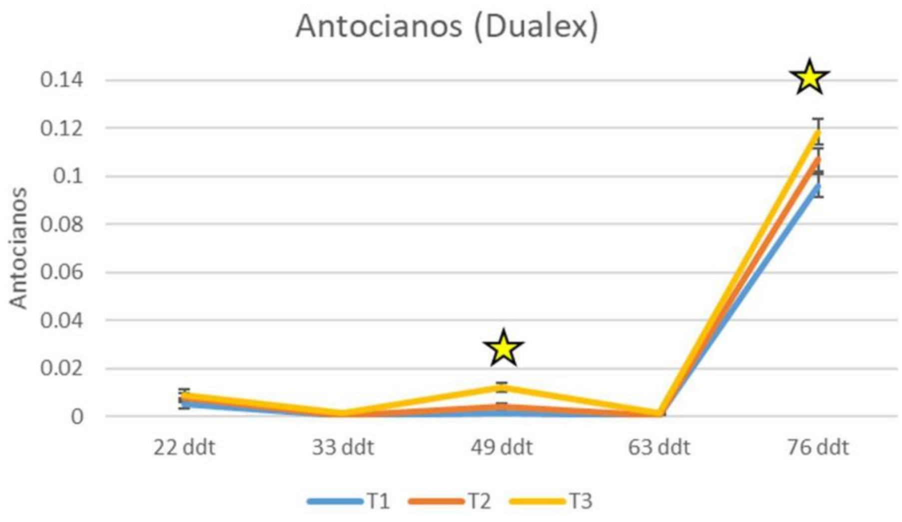

Figure 15. Evolution of the anthocyanin index measured with Dualex throughout the culture cycle in the different treatments. Each point is the average of the fourrepeats per treatment and its standard error. The stars indicate significant differences between treatments.

The NBI ratio defined as the ratio between chlorophyll-flavonoid index indicates that at 22 and 76 ddt there are no differences between treatments (Figure 16 and Table 10). The lowest NBI ratio occurred in T3 and with significant differences with T1 and T2 at 33 ddt and 63 ddt and only with T2 at 49 ddt.

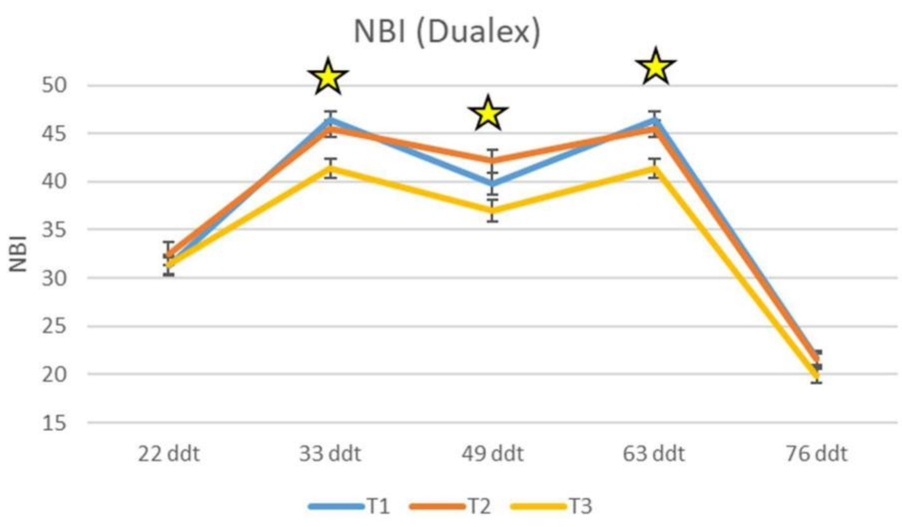

Figure 16. Evolution of the NBI index measured with Dualex throughout the crop cycle in the different treatments. Each point is the average of the four repetitions per treatment and its standard error. The stars indicate significant differences betweentreatments.

Table 10. Evolution of chlorophyll, flavonoid, anthocyanin and NBI indices measured with Dualex throughout the crop cycle in treatments T1, T2 and T3. The average of the four repetitions per treatment and their standard error are indicated . Different letters in the same column imply significant differences, "*": Significant differences p<0.05 and "**": Significant differences p<0.01, according to the Tukey-b test.

Chlorophylls (Dualex)											
Treatments		22 ddt		33 ddt		49 ddt		63 ddt		76 ddt	
T1	Average	34.01	b	39.12	a	39.51	a	39.12	a	27.00	b
	EE	0.6279		0.5046		0.5310		0.5089		0.4947	
T2	Average	37.29	a	39.37	a	40.57	a	39.37	a	28.74	a
	EE	0.8694		0.3416		0.4208		0.3416		0.5057	
T3	Average	37.51	a	38.19	a	37.51	b	38.19	a	26.99	b
	EE	0.7432		0.5123		0.5111		0.5123		0.5444	
Signification		**		**		**		**		*	

Flavonoids (Dualex)											
Treatments		22 ddt		33 ddt		49 ddt		63 ddt		76 ddt	
T1	Average	1.13	b	0.87	b	1.05	a	0.86	b	1.34	b
	EE	0.0158		0.0123		0.0186		0.0124		0.0264	
T2	Average	1.19	a	0.89	b	1.03	a	0.89	b	1.42	Off
	EE	0.0192		0.0139		0.0209		0.0139		0.0268	
T3	Average	1.24	a	0.96	a	1.10	a	0.96	a	1.47	a
	EE	0.0205		0.0143		0.0263		0.0143		0.0294	
Signification		*		**		**		**		**	

Antocianos (Dualex)											
Treatments		22 ddt		33 ddt		49 ddt		63 ddt		76 ddt	
T1	Average	0.0054	a	0.0006	a	0.0015	b	0.00059	a	0.0961	b
	EE	0.0021		0.0003		0.0008		0.0003		0.0049	
T2	Average	0.0079	a	0.0006	a	0.0045	b	0.00063	a	0.1070	Off
	EE	0.0019		0.0002		0.0013		0.0002		0.0048	
T3	Average	0.0091	a	0.0015	a	0.0123	a	0.0015	a	0.1186	a
	EE	0.0025		0.0005		0.0017		0.0005		0.0051	
Signification		**		**		**		**		**	

NBI (Dualex)											
Treatments		22 ddt		33 ddt		49 ddt		63 ddt		76 ddt	
T1	Average	31.23	a	46.37	a	39.72	Off	46.43	a	21.70	a
	EE	0.8636		0.8959		1.1277		0.9017		0.7692	
T2	Average	32.52	a	45.51	a	42.14	a	45.51	a	21.54	a
	EE	1.1788		0.8813		1.1898		0.8813		0.6973	
T3	Average	31.35	a	41.31	b	36.96	b	41.31	b	19.81	a
	EE	1.0619		0.9919		1.1094		0.9919		0.7045	
Signification		**		**		**		**		**	

7.d. Foliar analysis

The nutritional status of tomato leaf cultivation was determined at 64 ddt. Fully developed leaves were collected and located at the third node from the apex of the branch. In the laboratory to condition the sheets for analytical determinations, a washing protocol was followed first in soapy water without phosphates, rinsed in tap water and later in deionized water. They were dried in a forced ventilation stove at 65 º C, grinding and analytical determinations of N, P, K, Ca, Mg, Na, Fe, Cu , Mn and Zn were carried out in the General Laboratory of the Center of the Institute of Agricultural Research Finca La Orden- Valdesequera.

The results obtained have been compared with the interpretation table of foliar analysis for tomato industry, published by the Junta de Extremadura in 1992.

Table 11. Nutrient levels in leaves published by the Junta de Extremadura in 1992.

Niveles de nutrientes en hoja			
Nutriente	**Bajo**	**Normal**	**Alto**
N [%]	< 2.0	3.0 - 4.0	> 5.0
P [%]	< 0.1	0.2 - 0.35	> 0.6
K [%]	< 2.0	2.7 - 3.5	> 5.0
Ca [%]	< 2.0	2.0 - 3.5	> 4.5
Mg [%]	< 0.4	0.6- 1.0	> 2.0
S [%]	---	0.2 - 0.4	---
Fe [ppm]	< 80	100 - 150	> 175
Mn [ppm]	< 30	40 - 350	> 2500
Cu [ppm]	< 5	10 - 20	> 30
Zn [ppm]	< 15	20 - 60	> 75
B [ppm]	< 15	21 - 80	> 100

Table 12. Concentration of macronutrients (N, P, K, Ca and Mg) in tomato leaves. The average of the four repetitions per treatment and their standard error are indicated. Different letters in the same column imply significant differences, "*": Significant differences p<0.05 and "**": Significant differences p<0.01, according to the Tukey-b test. In green, the concentrations that are deficient with respect to the levels of nutrients in leaf published by the Junta de Extremadura are indicated.

Tratamientos		N (%)		P (%)		K (%)		Ca (%)		Mg (%)	
T1	Promedio	3.00	a	0.20	a	1.78	a	3.35	a	0.83	a
	EE	0.1291		0.0000		0.0250		0.0645		0.0250	
T2	Promedio	3.00	a	0.20	a	1.58	a	3.20	a	0.83	a
	EE	0.1080		0.0000		0.1031		0.1780		0.0250	
T3	Promedio	2.58	a	0.20	a	1.65	a	3.20	a	0.83	a
	EE	0.1031		0.0000		0.1500		0.1291		0.0250	
Significación		**		**		**		**		**	

There are no significant differences in the concentrations of N, P, K, Ca and Mg and they are all within the normal range , except for K which in all treatments are deficient (Tables 11 and 12).

Table 13. Concentration of micronutrients (Na, Fe, Cu, Mn and Zn) in tomato leaves. The average of the four repetitions per treatment and their standard error are indicated. Different letters in the same column imply significant differences, "*": Significant differences p<0.05 and "**": Significant differences p<0.01, according to the Tukey-b test. In red color indicates the concentration that is surplus with respect to the levels of nutrients in leaf published by the Junta de Extremadura.

Tratamientos		Na (ppm)		Fe (ppm)		Cu (ppm)		Mn (ppm)		Zn (ppm)	
T1	Promedio	2720.08	a	173.88	a	20.73	a	128.60	a	32.55	ab
	EE	142.7069		1.5580		0.7609		8.9289		1.6805	
T2	Promedio	2634.15	a	172.03	a	18.45	a	113.35	ab	29.05	b
	EE	420.7033		3.6181		1.1288		4.5787		1.0966	
T3	Promedio	1638.68	b	190.10	a	21.10	a	95.40	b	37.48	a
	EE	143.9578		10.0104		0.7937		3.7379		2.5418	
Significación		*		**		**		*		*	

There are also no significant differences in Fe and Cu concentrations (Tables 11 and 13) and there are excesses of Fe in T3 treatment. Significant differences were shown between T3 with T1 and T2 in Na, in Mn between T1 and T3 and in Zn between T3 and T2.

8. PRODUCTION AND QUALITY

9 sampling points were collected from the two central beds per treatment, determining the number and total weight of healthy fruits and waste (green and rotten), calculating commercial production in kg/ha. Quality parameters were determined by crushing 20 samples of healthy fruits.

Figure 17 and Table 14 show commercial production in kg/ha and significant differences between treatments. The T2 treatment was the one with the highest production, followed by T1 and the T3 treatment was the one with the lowest production and with significant differences with the treatments in which Phyto-C_3™ was added.

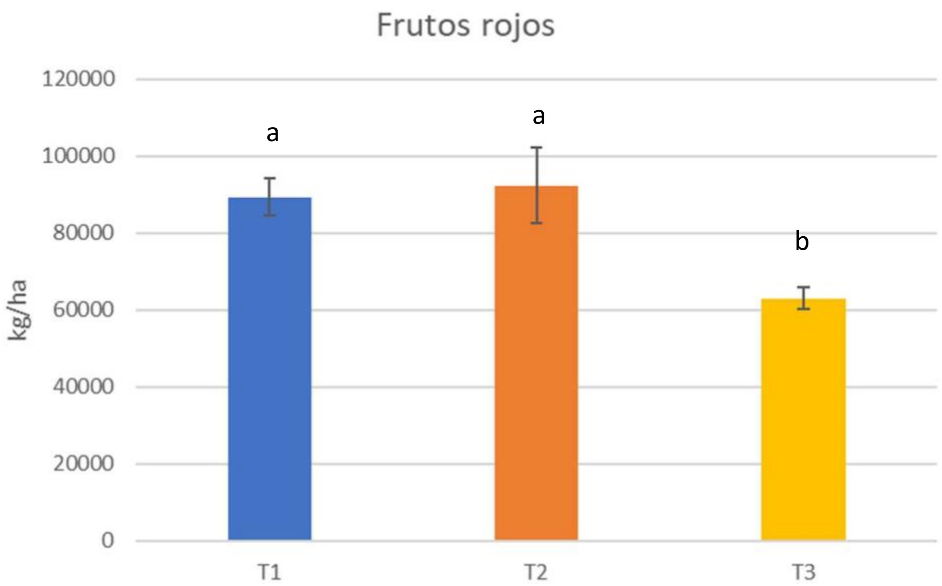

Figure 17. Production of red tomato fruits from the industry of each of the treatments in kg/ha. Each point is the average of the nine points per treatment and its standard error.

Regarding the kg/ha of green and rotten fruits (Figure 18 and Table 14) there are no significant differences between any of the treatments, however, it is observed that the treatment T2 was the one with the highest weight of green fruits and, T1 that of rotten fruits.

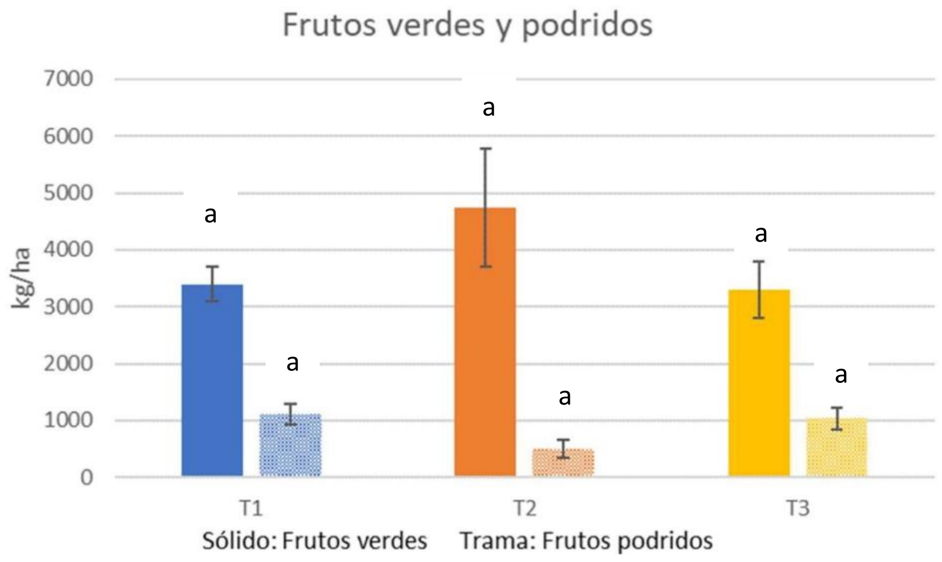

Figure 18. Production of green and rotten tomato fruits of each of the treatments in kg/ha. Each point is the average of the nine points per treatment and your error isgiven.

Table 14. Production of red, green and rotten tomato fruits in the different treatments. The average of the nine points per treatment and its standard error are indicated. Different letters in the same column imply significant differences, "*": Significant differences p<0.05. "**": Significant differences p<0.01, according to the Tukey-b test.

Production (kg/ha)							
Treatments		**Red Fruits**		**Green Fruits**		**Rotten fruits**	
T1	**Average**	**89246**	**a**	**3400**	**a**	**1107**	**a**
	EE	**4843.8670**		**310.7182**		**187.3921**	
T2	**Average**	**92300**	**a**	**4741**	**a**	**501**	**a**
	EE	**9867.4141**		**1044.8531**		**166.4677**	
T3	**Average**	**62982**	**b**	**3291**	**a**	**1036**	**a**
	EE	**2920.1963**		**497.5346**		**196.4953**	
Signification		*		**		**	

The number of red, green and rotten fruits is detailed in Figure 19 and 20 and Table 15. There are only significant differences in the number of red fruits between the T3 treatment and the Phyto-C_3™ treatments where T3 was the treatment with the lowest number of fruits and T1 with the highest number of fruits. There are no significant differences in the number of green and rotten fruits although it is observed that T3 was the treatment with the lowest number of green fruits.

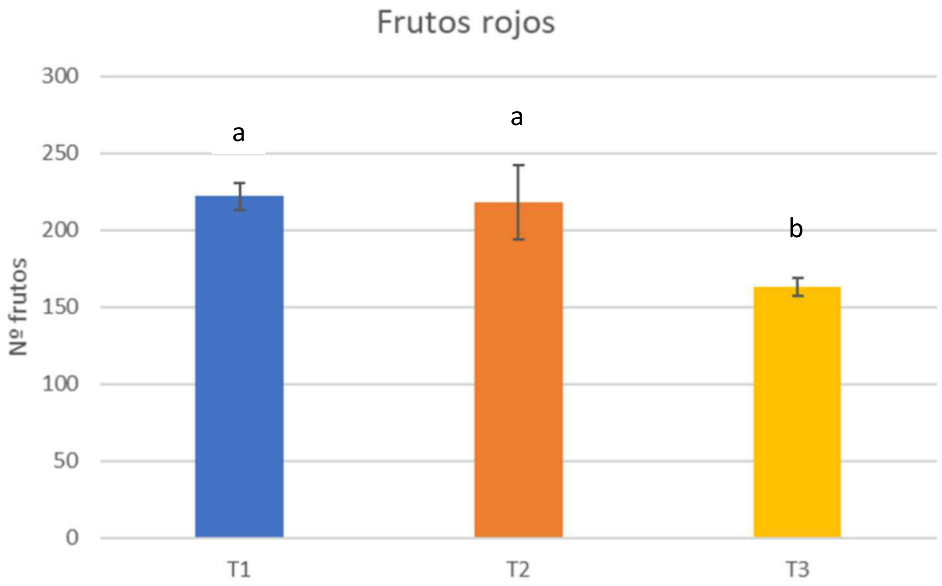

Figure 19. Number of red tomato fruits of each of the treatments. Each point is the average of the nine points per treatment and its standard error.

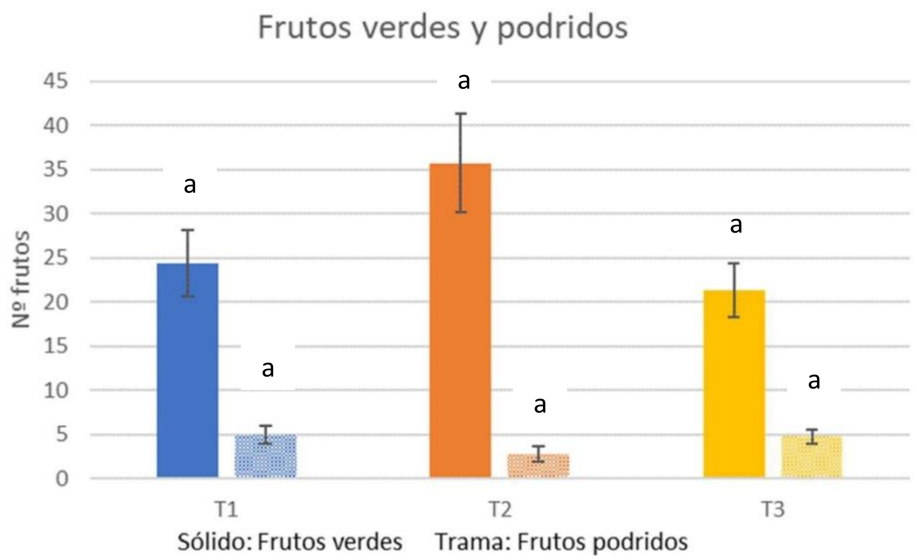

Figure 20. Number of green fruits and tomato drio of each of the treatments. Each point is the average of the nine points per treatment and its standard error.

Table 15. Number of red, green and rotten tomato fruits in the different treatments. The average of the nine points per treatment and its standard error are indicated. Different letters in the same column imply significant differences, "*": Significant differencesp<0.05. "**": Significant differences p<0.01, according to the Tukey-b test.

Number of fruits							
Treatments		**Red Fruits**		**Green Fruits**		**Rotten fruits**	
T1	**Average**	**222**	**a**	**24**	**a**	**5**	**a**
	EE	**8.9377**		**3.7680**		**1.0138**	
T2	**Average**	**218**	**a**	**36**	**a**	**3**	**a**
	EE	**23.8397**		**5.5221**		**0.8296**	
T3	**Average**	**163**	**b**	**21**	**a**	**5**	**a**
	EE	**6.0341**		**3.0641**		**0.7954**	
Signification		*		**		**	

For the determination of the quality parameters, the content of soluble solids (^{or}Brix), pH, acidity, color (L, a, b) on crushed sample of commercial tomato and specific weight has been evaluated.

Regarding the quality parameters such as soluble solids content, pH and % citric acid, there are no significant differences between treatments (Figures 21, 22 and 23 and Table 16) in which the T2 treatment was the one that presented lower ^{or}Brix and T1 lower % citric acid. Within the color parameters (Figures 24, 25 and 26 and Table 17) there are significant differences between T1 and treatments T2 and T3 in "HL" and "b", in parameter "a" there are no significant differences between treatments and the lowest value is presented by treatment T2.

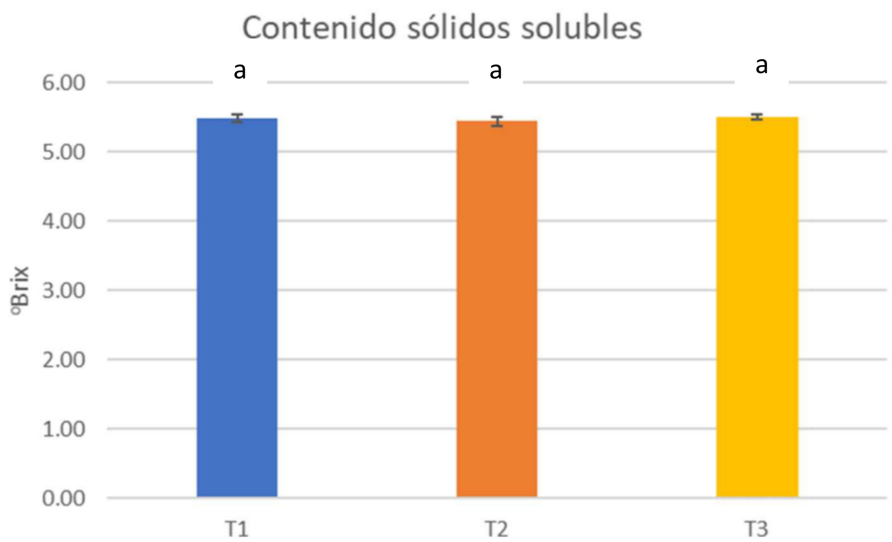

Figure 21. Content of soluble solids of each of the treatments. Each point is the average of the nine points per treatment and its standard error.

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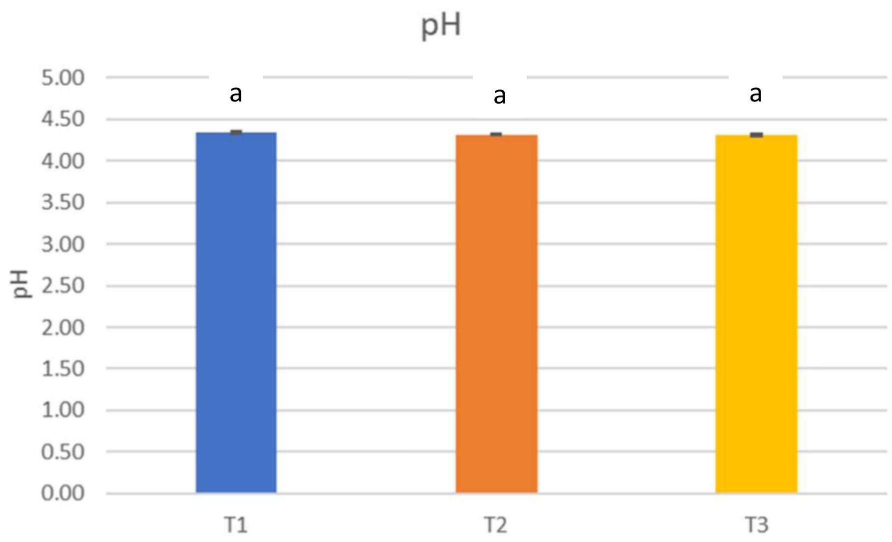

Figure 22. pH of each of the treatments Each point is the average of the nine points per treatment and its standard error.

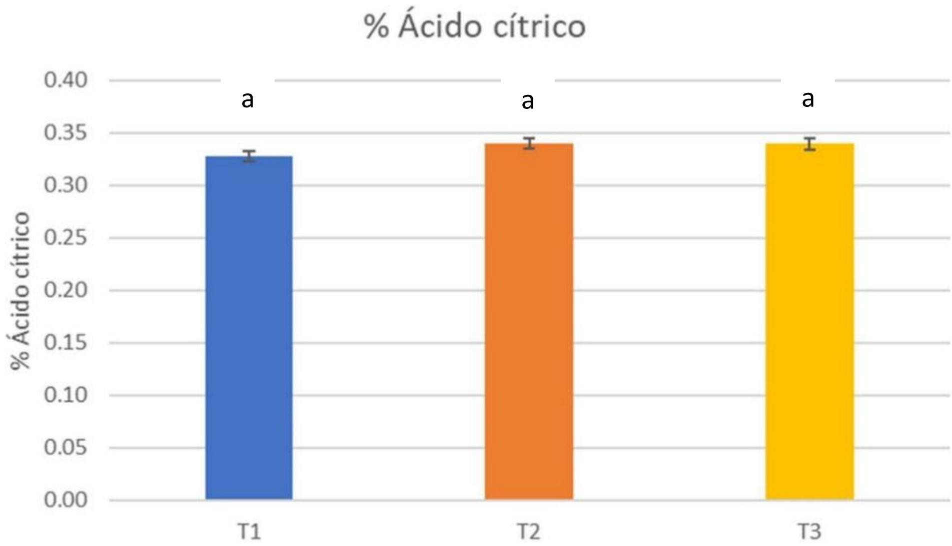

Figure 23. Citric acid content of each of the treatments. Each point is the average of the nine points per treatment and its standard error.

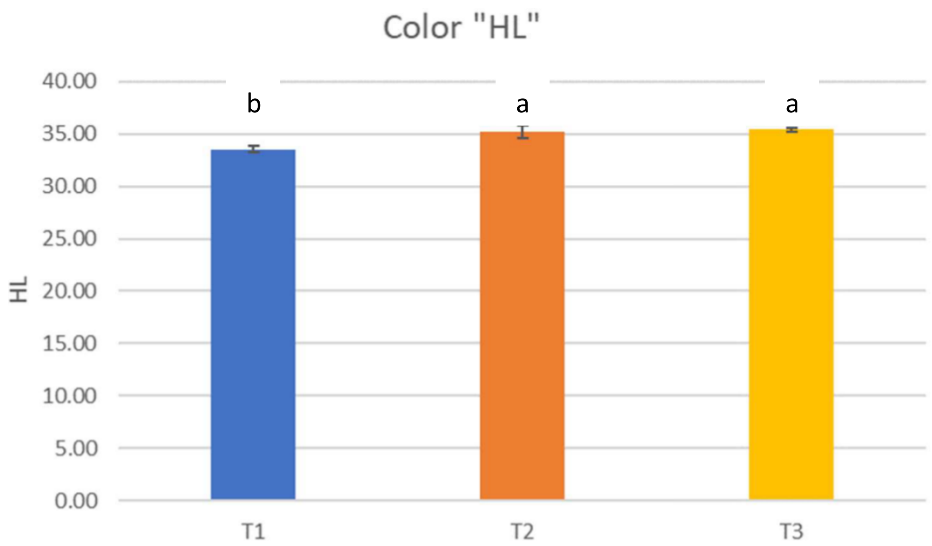

Figure 24. Color (LH parameter) of each of the treatments. Each point is the average of the nine points per treatment and its standard error.

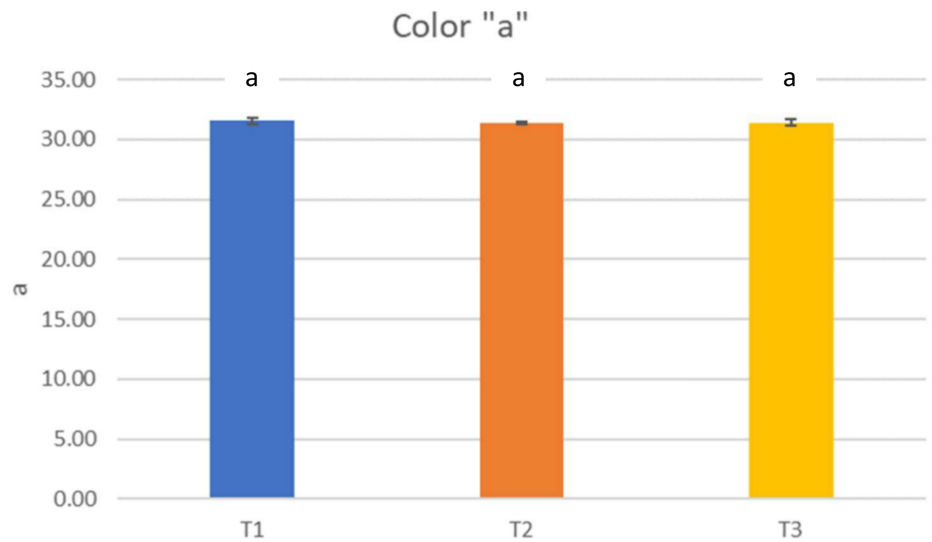

Figure 25. Color (parameter a) of each of the treatments Each point is the average of the nine points per treatment and its standard error.

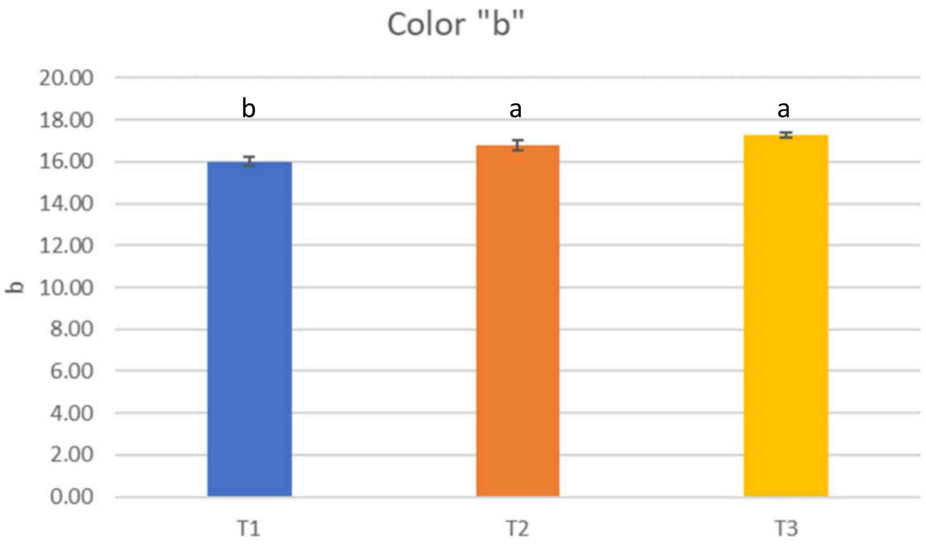

Figure 26. Color (parameter b) of each of the treatments. Each point is the average of the nine points per treatment and its standard error.

Table 16. ºBrix, pH and % citric acid in T1, T2 and T3. The average of the nine points per treatment and its standard error are indicated . Different letters in the same column imply significant differences, "*": Significant differences p<0.05. "**": Significant differences p<0.01, according to the Tukey-b test.

Quality parameters							
Treatments		the Brix		pH		% citric acid	
T1	Average	5.49	a	4.34	a	0.328	a
	EE	0.0582		0.0126		0.0051	
T2	Average	5.27	a	4.32	a	0.340	a
	EE	0.0963		0.0066		0.0047	
T3	Average	5.50	a	4.31	a	0.340	a
	EE	0.0343		0.0119		0.0056	
Signification		**		**		**	

Table 17. Color parameters HL, a and b in T1, T2 and T3. The average of the nine points per treatment and its standard error are indicated . Different letters in the same column imply significant differences, "*": Significant differences p<0.05. "**": Significant differences p<0.01, according to the Tukey-b test.

Quality parameters							
Treatments		Color "HL"		Color "a"		Color "b"	
T1	Average	33.52	b	31.54	a	16.02	b
	EE	0.3043		0.3086		0.2404	
T2	Average	35.20	a	31.40	a	16.76	a
	EE	0.6154		0.1149		0.2472	
T3	Average	35.41	a	31.43	a	17.25	a
	EE	0.2157		0.2747		0.1214	
Signification		**		**		**	

For the determination of the weight specific herself Weighed 10 Fruits Red y Green in fresh y were dried in a forced-ventilated stove at 65ºC to constant weight. The results are shown in Figures 27 and 28 and Table 18. It is observed that there are no significant differences in the fresh weight y dry of 10 fruits red and Green atnque herself notes that the 10 fruits Red of T2 Weighed more What the remainder of the Treatments y in T3 Were the Fruits Green.

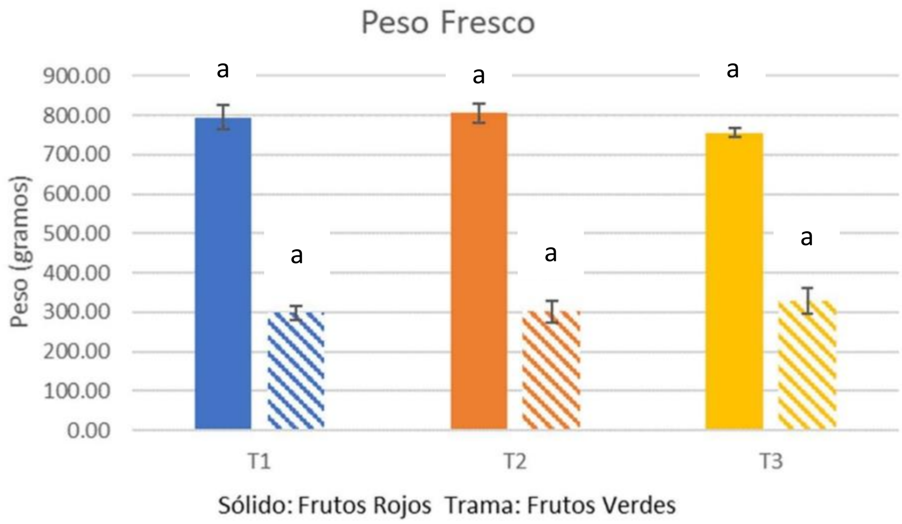

Figure 27. Fresh weight of 10 red and green fruits of each of the treatments. Each point is the average of the four repetitions per treatment and its standard error.

Figure 28. Dry weight of 10 red and green fruits of each of the treatments. Each point is the average of the four repetitions per treatment and its standard error.

Table 18. Fresh and dry weight in 10 berries and 10 green fruits in T1, T2 and T3. The average of the four repetitions per treatment and their standard error are indicated. Different letters in the same column imply significant differences, "*": Significant differences p<0.05. "**": Significant differences p<0.01, according to the Tukey-b test.

Treatments		10 Red Fruits				10 Green Fruits			
		Fresh Weight		Dry Weight		Fresh Weight		Dry Weight	
T1	Average	794.54	a	51.17	a	297.88	a	20.92	a
	EE	30.5307		2.1618		17.5071		1.3411	
T2	Average	806.15	a	52.84	a	301.50	a	20.06	a
	EE	24.1621		1.7284		26.9903		1.8649	
T3	Average	755.86	a	47.88	a	329.01	a	21.33	a
	EE	18.0523		0.8971		12.5049		0.9205	
Signification		**		**		**		**	

10. EFFECT OF PHYTO-C_3™ PRODUCTS ON THE INSIDE OF THE IRRIGATION BELT

Once the fruits were collected, the effect of Phyto-C_3™ products on the irrigation belt was evaluated. 5 m of irrigation tape were taken from each of the treatments and in the laboratory they were discovered leaving the interior in sight. It was observed that the interior of the irrigation belt (Figure 29) of the T3 treatment (control) is the one that presented the highest amount of residues while the one with the least residues was T2 (organic Phyto-C_3™).

T1 **T2** **T3**

Figure 29. Interior of the irrigation pipes after collection of treatments T1, T2 and T3.

11. CONCLUSIONS

- Although the electrical conductivity of the soil samples collected both in pre-transplantation and at the end of the crop indicate that the soil is non-saline, it is observed that at the end of the crop there is an increase in this parameter in all treatments.

- The content of P (ppm) in soil is high both at the beginning and at the end of the crop, however, it is observed that in T1 and T2 they decrease and in the control treatment (T3) they increase after collection with respect to the pre-transplant sampling.

- The K content (meq / 100g) in both T1 and T2 treatments in both samples is deficient and while in T1 a slight increase is observed, in T2 a decrease in this element is observed. On the contrary, in the control treatment (T3) it goes from being elevated to definitive.

- The evolution of the Na content (meg/100g) in soil passes towards normal values from pre-transplant to the end of cultivation in T1 and T2 treatments.

- The concentration of Ca (meq/100g) at 107 ddt is within the range of normality, however, in pre-transplantation it presented deficiencies in T1 and excesses in T3.

- At the end of the crop, the amount of Mg (meq/100g) present in the soil is high and exceeds the normal range and, while in the control treatment or without application of Phyto-C_3™ (T3) it is maintained at similar concentrations in both samplings, in T1 and T2 there is a considerable increase.

- To the Start of cultivation the content of $N\text{-}NO_3^-$ (kg/ha) and N- total (kg/ha) of the treatment T3 (control) is lower than T1 and T2 and with significant differences. The first application of the various products of Phyto-C_3™ were performed 16 days later so we cannot attribute the significant difference found with the application of Phyto-C_3™ American u organic but What Puts of manifest the heterogeneity of the soil. Thorough sampling is an essential task in determining the chemical composition of the plot.

- After gathering the form chemistry Majority Taken in the soil it $N\text{-}NO_3^-$(kg/ha). T1 treatment has the highest levels of $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ (kg/ha) on the contrary, T3 is the one with the lowest content of both chemical forms.

- All treatments had the same fertilization plan and although the chemical composition of the different Phyto-C_3™ products and their effect on the soil is unknown , it is observed that the T1 treatment (American Phyto-C_3™) at the end of the crop has a higher content of N provided in the form of nitrates.

- Between the initial and final sampling of soil, the content of $N\text{-}NO_3^-$ (kg / ha) is increased in all treatments mainly in T1, the contents of $N\text{-}NH_4^+$ decrease in T2 and T3 while in T1 also increase.

- The concentration of the different chemical forms in irrigation water are variable in the different samplings carried out. It is useful to perform periodic analyses to determine the N content, mainly if well water is irrigated.

- In this work, the NDVI index shows more sensitivity for nutritional diagnosis than the NDVIG index. In the early stages of the vegetative cycle, there are no differences between treatments with Phyto-C_3™ but there are differences between these with control treatment. Until 33 ddt, at which time three applications of the test products had been made, no differences between T1 and T2 begin to be observed and from that date the T1 treatment is the one that shows more values high in the NDVI index, except at 49 ddt.

- The SPAD index only allowed to establish significant differences in two phenological stages of the crop. Between treatments with Phyto-C_3™ there are no significant differences throughout the development of the crop. The control treatment (T3) from 33 ddt presented a lower SPAD index than the treatments with Phyto-C_3™ (T1 and T2 and with significant differences in this phenological state and at 76 ddt . The results obtained with this index indicate that the control treatment was the one with the worst nutritional status throughout the crop.

- The chlorophyll index measured with the Dualex equipment indicates that T2 (organic Phyto-C_3™) was the one that showed the highest values from 33 ddt and the lowest were presented by T3 (control treatment).

- The index of flavonoids and anthocyanins indicate that the T3 treatment (control) had the highest index, while T1 had the lowest index. The synthesis of these compounds is linked to an imbalance in cellular functioning and are indicators that plants are under some type of stress. All treatments were fertigated in the same way and were subjected to the same environmental conditions, the differences resided in the type of product applied of Phyto-C_3™ compared to the control that did not lead to application of Phyto-C_3™.

- The NBI ratio (chlorophylls / flavonoids) was lower for the T3 treatment and the highest was different according to the phenological state analyzed.

- There are no significant differences in the concentration of N, P, K, Ca, Mg, Na, Fe and Cu. The concentration of K is d efficient in all treatments and that of Fe in T3 treatment is in excess. There are only significant differences between T3 with T1 and T2 in Na, in Mn between T1 and T3 and in Zn between T3 and T2.

- The T3 treatment had the lowest production of commercial tomatoes and with significant differences with the Phyto-C_3™ treatments. The treatment with the highest commercial production was for T2.

- The production of green and rotten fruits did not present significant differences between treatments however, in the weight of green fruits would be expected to find them but the difference in weight in the different sampling points of the T2 treatment is the cause that is not shown. The treatment with higherr weight of green fruits was T2 and that of rotten fruits T1.

- There were no significant differences between treatments in soluble solids content, pH, % citric acid and parameter "a" in color. The lowest value of oBrix was presented by the T2 treatment and the least acid was given in T1.

- There were no significant differences in the fresh and dry weight of10 red and green fruits between treatments, although it was observed that the T2 treatment had a higher weight of red fruits and T3 that of a lower weight. In green fruits, the heaviest was the T3 treatment (control).

- The T3 treatment was the one that presented the greatest amount of residues inside the irrigation belt and considering the treatments with Phyto-C_3™, T2 had the presence of less residues than T1.

In view of the results we can say:

-The T1 treatment (Phyto-C_3™ American) presented a higher N content in soil after harvesting, shows higher values of the NDVI index and lower values in flavonoids and anthocyanins, greater number of red fruits and lower % citric acid.

-The T2 treatment (organic Phyto-C_3™) had the highest chlorophyll index measured with Dualex, it is the treatment with the highest production of red and green fruits, lower content of soluble solids, with the highest average specific weight of 10 red fruits and less presence of residues in the irrigation belt.

-The T3 treatment (Control or without application of Phyto-C_3™) presented the chlorophyll index measured with SPAD Minolta 502 and Dualex lower, high levels of flavonoids and anthocyanins, lower NBI ratio, excess Fe in leaf, lower yield in red fruits both by weight and number of fruits, greater weight in 10 green fruits and the irrigation belt with the highest amount of residues.

12. BIBLIOGRAPHY

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