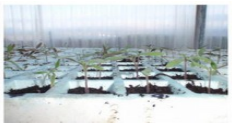
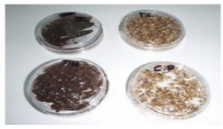
MICROALGAE BIO-STIMULATING EFFECT ON TRADITIONAL TOMATO AGRICULTURE

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Microalgae bio-stimulating effect on traditional tomato agriculture

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Abstract

Soil care, organic agriculture and preservation of the environment are among the current interests of sustainable agriculture. Numerous plants' stimulators are tested in the world as an alternative to organic agriculture. In the Canary Technology Institute (ITC) of Gran Canaria and under greenhouse conditions this study was carried out in order to analyze the effect of microalgae culture as irrigation water on *Solanum lycopersicum* plant. Through evaluations from days 7, 14, 21 and 28 the crop growth was analyzed. A bio-stimulating effect of *Chlorella pyrenoidosa and Chlorella sp* was established.

Keywords: *Chlorella pyrenoidosa and Chlorella sp*, Microalgae, Open ponds, *Solanum lycopersicum var. Charony*, Agriculture, Bio-stimulants, Seed, Substrate, Irrigation water.

Resumen

El cuidado del suelo, la agricultura orgánica y la conservación del medio ambiente son algunos de los intereses actuales de la agricultura sostenible. Estimuladores para plantas son analizados en el mundo como alternativa en la agricultura orgánica. En el Instituto Tecnológico de Canarias (ITC) de Gran Canaria y bajo condiciones de invernadero se llevó a cabo este estudio con el objeto de analizar el efecto del uso de un agua de riego procedente del cultivo de microalgas sobre el crecimiento en plantas de *Solanum lycopersicum*. Mediante evaluaciones realizadas a partir de los días 7, 14, 21 y 28 se analizó la dinámica de crecimiento del cultivo. Un efecto bio-estimulante procedente del uso de Chlorella pyrenoidosa y Cholrella sp fue determinado.

Palabras claves: *Chlorella pyrenoidosa and Chlorella sp* , Microalgas, Raceways, *Solanum lycopersicum var. Charony*, Agricultura, Bio-estimulantes, Sustrato, Cytokininas, Agua de riego.

INDEX

1. INTRODUCTION

Seaweeds have been applied as soil conditioning agents for decades (Chapman and Chapman 1980; Guiry and Blunden 1981; Blunden 1986; Temple and Bomke 1988; Metting et al. 1988, 1990; Hong et al. 2007). About 15 million metric tonnes of seaweed products are produced annually (FAO 2006). A considerable portion of them is used as bio-stimulants to increase plant growth and yield. Numerous studies have revealed a wide range of beneficial effects of seaweed extract applications such as improved crop performance and yield, elevate resistance to biotic and abiotic stress, enhance postharvest shelf-life of perishable products, etc. (Hankins and Hockey 1990; Blunden 1991; Zhang and Schmidt 1997; Norrie and Keathley 2006). Besides eliciting a growth-promoting effect on plants, seaweeds also affect the properties of soil which in turn, influence plant growth (Jeannin and others 1991; Eyras and others 1998; Gandhiyappan and Perumal 2001; Moore 2004 ;). Although many of the chemicals components of seaweed extracts and their modes of action remain unknown (Fornes and others 2002; Vernieri and others 2005).

The beneficial effects of Cyanophyta on improving the growth and yield of the crop plants cannot be explained by elevated nitrogen levels alone (Boussiba, 1988; Whitton, 2000). It has been suggested that plant hormones synthesized by Cyanophyta may also play a role in promoting growth (Pedurand and Reynaud 1987). It is probably that secondary metabolites present in the Cyanophyta such as cytokinins, will also be released into the soil during cell decomposition and so, will become available for uptake by plant roots. The cytokinins effect on various physiological processes is well documented with many practical applications in tissue culture and agriculture (Mok 1994; Kamı´nek et al. 1997; Schmu¨lling, 2002; Sakakibara 2006). Cytokinins have also been identified in Chlorophyta. Cytokinin-like activity was measured in strains Chlorella sp. and others isolated from soil samples collected in Brazil (Stirk et al. 2002; O¨ rdo¨g et al. 2004). The diversity of cytokinin forms detected, in vitro deuterium-labeling experiments and others studies related with cytikinin-like activity into their growth media, suggest that microalgae are able to synthesis their own cytokinins (Stirk et al. 2006; Burkiewicz 1987).

Plants grown in soils treated with seaweed or extracts applied either to the soil or foliage, exhibit a wide range of responses that have been well documented. Positive responses include improved germination, root development, leaf quality, general plant vigor and resistance to pathogens (Myklestad 1964; Booth 1965, 1966, 1969, 1981; Stephenson 1974; Blunden 1977; Senn and Kingman 1978; Abetz 1980; Verkleij 1992; Kahn et al. 2009).

Tomato “Solanum Lycopersicum” is considered one of the most important vegetable in many countries (Nuez, F. 1995). In 1885 was established in Canary Island the first crops of tomatoes. Juliano Bonny Gómez S.L., in those early years, was one of the pioneers and promoters of tomatoes crop, essential, even our days, for Canary Island economy (EDEI, 1996). The recent challenges in food production due to the increasing occurrence of biotic and abiotic stress as likely due to climate change will further reduce yields and will have an impact on crops in the 21st century. Therefore, research into developing sustainable methods to alleviate these stresses should be a priority (IPCC 2007).

Bio-stimulants used in tomato crops have accomplished effects on root development by improving lateral and longitudinal root formation, roots nutrient uptake, increasing total volume and vigor of the root system, plant chlorophyll content enhanced so at the same time general plant growth (Finnie and van Staden 1985; Crouch and van Staden 1992; Atzmon and Van Staden 1994; Blunden and others 1997; Thompson 2004; Sla`vik 2005; Mancuso and others 2006). However, bio-stimulants are unable to provide all the nutrients needed by a plant in required quantities (Schmidt and others 2003). Studies with radiolabelled tomato seedlings show clear evidence that roots are able to take up cytokinins from their external environment, metabolize them and then release some back into the growth medium (Van Staden 1976b ;Arthur et al. 2001a). High quantity of microalgae biomass is produced to cover industry demand and several systems of microalgae culture are used and studied for culturing in an extensive scale. Photo-biorreactors are closed systems where microalgae are growing under artificial, natural or combined illumination. They have been successfully used for producing large quantities of microalgae biomass (Molina Grima et al., 1999; Tredici, 1999; Pulz, 2001; Carvalho et al., 2006). Open pond (raceways) are made of a closed loop recirculation channel, built in concrete blocks or compacted earth. Mixing and culture circulation is provided by the

paddlewheels. Microalgae cultures of raceways are exposed under atmospheric or greenhouse conditions where the temperature fluctuates during the diurnal cycle and the seasons (Richmond A., 1991). Microalgae are relatively easily grown in mass culture and they provide a source of biological material with potential application in agricultural industries. (Moore, 2004)

In an attempt to develop a cost-effective methodology for *Chlorella sp* mass cultivation, the company Juliano Bonny Gómez S.L. is studying the *Chlorella* culture in open systems (raceways) to obtain high value synergies between traditional tomato crop and microalgae culture. To reach this goal Juliano Bonny Gómez S.L. and the Canary Technological Institute (ITC) signed a collaboration agreement to develop a new project: "Feasibility study on microalgae culture as a complementary activity to traditional agriculture", based on a previous ITC project called MAXPRUA. *Chlorella pyroneidosa and Chlorella sp* cultures were placed in the southeast of Gran Canaria (Canary Island), in the pilot plant facilities of the biotechnology department of the Canary Technological Institute (ITC). These organisms provide the author with their support, knowledge and facilities for the development of her research.

The aim of this paper is to show the first results of a series of tests carried out with the intention to endorse the hypothesis of a possible bio-stimulating effect in tomato crop using *Chlorella pyr and Chlorella sp* culture growth media as irrigation water.

2. OBJECTIVES

1. Demonstrate the possibility of bio-stimulant effect on *Solanum lycopersicum* plant using microalgae culture growth media as irrigation water.
2. To identify possible different bio-stimulant affects between strains of *Chlorella pyrenoidosa*) and *Chlorella sp.* (Hydroponic water isolated).
3. To analyze behavior differences between two substrates (peat and vermiculite) under the bio-stimulant influence.

3. MATERIALS AND METHODS

3.1. Study location

The study was conducted in March-May of 2010 at the Canary Technological Institute (ITC) within the premises of the Biotechnology's Department, located in Pozo Izquierdo in Gran Canaria.

3.2. Biological material

- Tomato seeds *Solanum lycopersicum var. charony* was obtained from Juliano Bonny Gómez S.L.
- The strain of *Chlorella pyrenoidosa* (BNA-ITC-Chlo-pyr-01) and Chlorella *sp.* (Hydroponic water isolated) (ITC-Chlo-hidrop-09) were facilitated by the research group of ITC biotechnology department.
- As substrates, were chosen, Substrate T: peat (mixture of black and light pet; Juliano Bonny Gómez S.L.) as organic substrate and substrate V: vermiculite as mineral substrate (Vermiculita Extendida; Perindustria S.L.).

3.3. Irrigation water solutions

A = Water from *Chlorella Pyr* culture + macro and micro nutrients to reach 100% of its initial concentration according to modified prescription of Tamiya, 1968 recipe.

B = Water from *Chlorella hy* culture + macro and micro nutrients to reach 100% of its initial concentration according to modified prescription of Tamiya, 1968 recipe.

C = Water control (sterilized freshwater) + macro and micro nutrients to reach the same concentration according to chlorella supernatant ones.

3.4. Culture scale up process

3.4.1. Culture media

Culture was grown in filtered (0.2 μm) and sterilized freshwater (121ºC during 22 min), enriched with M/M modified medium (Table 1).

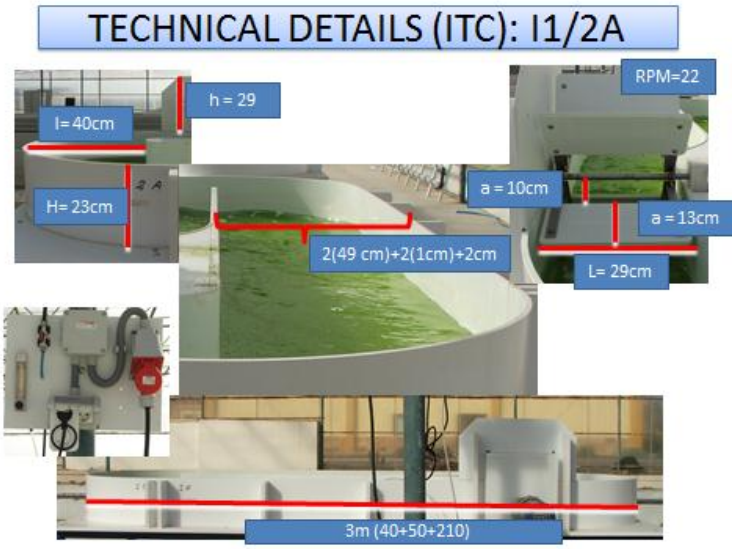
Table 1: M/M medium for laboratory culture.

SOLUTION 1 (g/L): (S.Beijerink) (25ml/L)	SOLUTION 2(g/L): (25ml/L)	MICRO-NUTRIENTS(g/l): (1ml/L)
$MgSO_4*7H_2O$ = 4.00g	KH_2PO_4 = 14.52g	$CoCl_2*$ $6H_2O$ = 1.61g
$CaCl_2$ = 1.54g	K_2HPO_4 = 37.42g	EDTA = 50.00mg
NH_4Cl = 16.00g.		$MnCl_2$ * $4H_2O$ = 5.06 g
		$CuSO_4$ * $5H_2O$ = 1.57g
		H_3BO_3 = 11.40g
		$ZnSO_4*7H_2O$ = 22.00g
		$(NH_4)Mo_7O_{24}$ = 1.10g
		$FeSO_4$ * $7H_2O$ = 4.99g

Recipients of different sizes were used for the scale up process. After being, sterilized, they were filled with the volume of MM medium necessary in each step, 24 h previously to the inoculation day. The scaling up was carried out from stock cultures of the growth chamber conducted in containers of 500ml borosilicate Erlenmeyer, containers were stirred with filtered air, enrichment with 0.3-0.5% CO_2 in air (V / V). The stock was maintained in exponential growth by sub culturing weekly series. (Carmona, D. L. 2007; Said, M.M. 2010). Laboratory cultures were grown under 20˚C temperature and 55% humidity conditions. Photon flux (PAR) of 250 mmol photon m^{-2} s^{-1} was continuously provided by fluorescent tubes (Philips Master TLD 36W/840).

3.4.2. Semi-continuous culture at a pilot plant scale

For open pond *Chorella pyr and Chlorella hy* production, cells were cultured in two 3 m^2 raceways, in a semi-continuous system. Raceways containing 250L of sterilized freshwater, with a UV-water sterilizer (INTER OZONO) into the greenhouse, enriched with Tamiya (1968) modified medium (Table 2) were filled with 8L chlorella culture Tamiya, (1968) medium scale up previously in the laboratory. Raceways were built with concrete blocks and covered with food grade quality PVC-lining (UV-resistant polyvinylchloride). Paddlewheel were turned at 22 r.p.m., providing enough mix to the water column, light and CO_2 (Picture 1).



Picture 1. 3m^2 raceway, with 250L Chlorella *sp,* growing under greenhouse conditions. 0.4 l min^{-1} CO_2 flux was injected during 1 hour, 5 times per day.

3.4.3. Harvesting process

During the harvesting process (Westfalia), 20% culture (1.0-1.3 optical density) was harvested 2 times per week but also 80% was harvested each weak to maintain semi-continuous culture. The culture volume removed was replaced with new f/2 medium each time. Once harvested two distinct fluids were collected, the culture supernatant, and the wet paste or biomass. Cells were continuously provided with nutrients supply.

Table 2: (Tamiya, 1968) f/2 modified medium for semi-continuous culture.

NUTRIENTS	CONCENTRATION	(mg/L)
KNO_3	2.50	
$MgSO_4$	1.25	
KH_2PO_4	0.63	CULTURE
SOLUTION	SOLUTION (g/L)	SOLUCION (ml/L)
SOLUTION A		
$FeSO_4$	273	0.1
EDTA	10	
SOLUTION B		
H_3BO_3	154	0.1
EDTA	6	
SOLUTION C		
$MnSO_4$	111.5	
$CuSO_4$	124.5	0.5
$ZnSO_4$	134.5	
$CoSO_2$	140.5	
$(NH_4)Mo_7O$	85	
EDTA	25	

Macronutrients were added directly as powder. Micronutrients were previously dissolved in distilled water. Culture growths were controlled daily. The optical density of the culture was measured by Omega UV-Vis spectrophotometer, at 750 nm (OD_{750}). pH and temperature were monitored by a

hand-pH meter (VWR pH100). Irradiance was recorded with Lycor (LI 1400) radiometer. Salinity was measured with a hand-refractometer (Atago) and it was maintained in 0-1 ‰. Number of cells was quantified by a counting chamber (Neubauer) and an Olympus microscope model CH 30.

3.5. Obtaining Irrigation water

Two supernatant mentioned earlier were obtained, during the harvesting process, one from *Chlorella pyr* culture and the other from *Chlorella hy* besides these two supernatants, control supernatant was also used as irrigation water. To assess the potential bio-stimulating effect microalgae culture in the tomato crop, only one unknown variable was necessary. So, to carry this premise particular treatments were required to the supernatants obtained. Nutrient concentration profiles were conducted analyzing the irrigation water every day for each one of the samples (MIcromac-1000 Systea) to characterize the water samples which allow us to evaluate which nutrient concentrations have been used every day by the microalgae for their development. Carry each watering day back to 100% of its initial concentration according to the initial modified prescription of Tamiya (1968) was performed, in order to equalize the nutrients concentrations. Three irrigation waters solutions (A, B & C) were obtained subsequently of the treatments.

3.6. Seed irrigation process

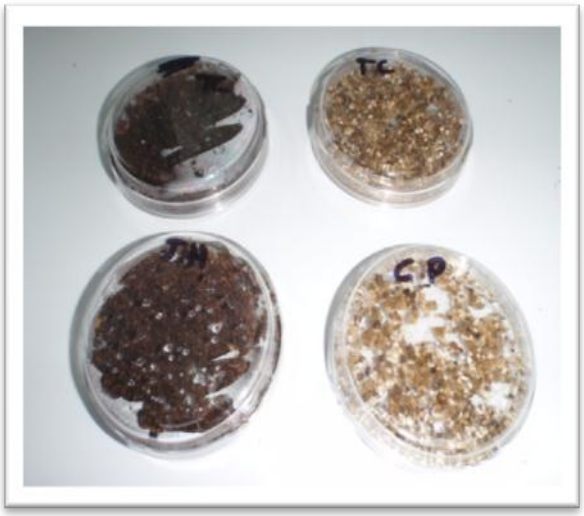
Once matched the concentrations of different irrigation water three showers 3-liter capacity, properly identified with irrigation water source were caught. Every day the identified seed were watered carefully to avoid splashing or holes. A total of 210 *Solanum lycopersicum var. charony* seeds were planted, 105 of the 210 seed per substrate (T & V) and 35 of the 105 for each irrigation water treatment (A-B-C) (Picture 2).

Seeds were kept for 28 days; highlighted three distinct phases:

Seeding phase, emergency phase and growth phase. Emergence, stem growth (length and width), dry weight and number of green leaves were evaluated. (Bewley, J et al. 1986; Castilla, N., 1995).

Picture 2. *Solanum lycopersicum* seeds

3.7. Seed treatment in Petri dishes



Picture 3. 15.5 x 2 cm petri dishes.

10 tomatoes seeds per plate were placed in 15.5 x 2 cm petri dishes, with its corresponding substrate. 10 ml aqueous solution of each treatment (A-B-C) was added in each plate (Picture 3). Plates were kept on camera during a period of 10 days with 12h light and 12h of darkness at 24+/- 1.5 ºC temperature (Killian, S. and M. Lewis, 2005). Germination percentage was determined.

4. RESULTS

4.1. Cell density curve

Chlorella pyr and Chlorella hy were grown from March to May 2010 in $3m^2$ raceway. Both cultures were grown under greenhouse conditions, with an average temperature of 23.39±2.01 for C*hlorella pyr* culture and 23.58±2.04 for C*hlorella hy* culture and natural illumination (586.28 µmol photons m^{-2} s^{-2}). pH was maintained at 8.03±0.46 for *Chlorella pyr* culture and at 8.17±0.35 for *Chlorella hy* culture, with 5 CO_2 injections per day during 1 hour (08h-17h). Semi-

continuous system process was started at day 10, when 80% cultures were harvested and the same volume fresh medium was replaced. After the first harvested day, the cultures were grown in exponential phase continuously, because they were harvested at high optical density (1-1.2). Harvested days are represented in the peaks curve, when the maximum optical density was reached. After the harvested process, new medium was added to provide nutrients and dilute the culture (Fig. 1).

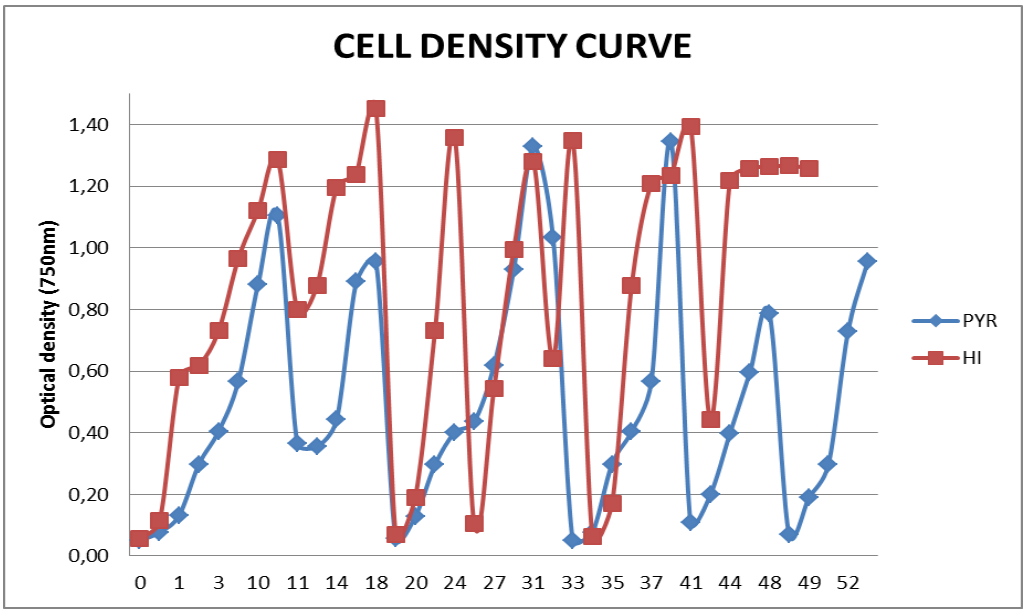


Figure 1. Cell density versus culture days of *Chlorella* cultures. *Chlorella pyr* is represented by blue line and red line represents *Chlorella hy.* Cell density increase until the harvesting days and decrease after culture dilution.

4.2. Calibration Curve

Calibration curve of cell density versus optical density was obtained with measurements of optical density and cell direct counting (Fig.2). Cell direct counting is considered a precise method but time consuming. Optical density is a turbimetric method very practical and easy to use. Absorbance measurements depend on the quantity matter and characteristics of culture. A calibration curve was calculated in the exponential culture phase, at a wavelength not corresponding with the maximal peak absorption (750nm), to avoid the influence of the culture conditions changes. Equations: PRY: y=439.9x – 36.563, R^2 =

0.9804 and HI: y=561.29x – 66.905, R^2 = 0.9716 indicates a correct value adjustment.

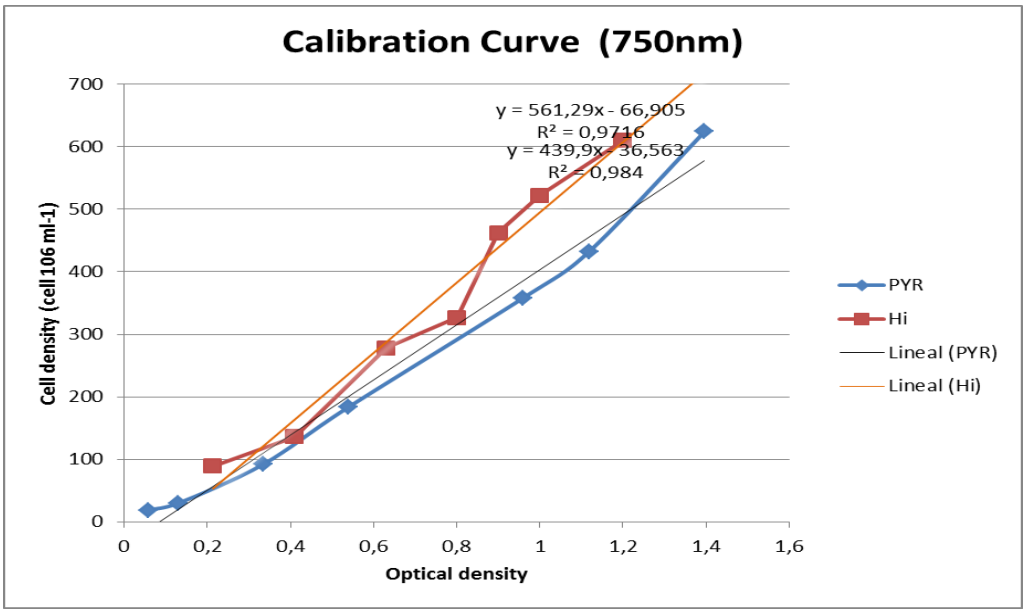


Figure 2. Calibration curve. Cell density versus optical density (750 nm). *Chlorella pyr* is represented by blue line and red line represents *Chlorella hy.*

4.3. Emergency % Analysis in Petri dishes

No differences between irrigation solution A and B were found in peat substrate (T), but almost 20% more emergency rate was found in both case in contrast with irrigation solution C. An emergency rate on day 3 almost twice that of irrigation solutions C results was found in seeds watered with irrigation solution A and B. For vermiculite substrate (V) no variances were found among the water source. However, seeds watered with A and B solutions present an emergency rate on day 3 of 6% and 16% respectively higher than the water control. The obtained results referring to the emergence % rate note than on day 3 in both substrates, water control had lower values. As for the final emergence percentage there weren't significants differences between treatments using vermiculite substrate. On the other hand in the case of peat substrate, there was a decrease in germination rate for irrigation water B (Figure 3).

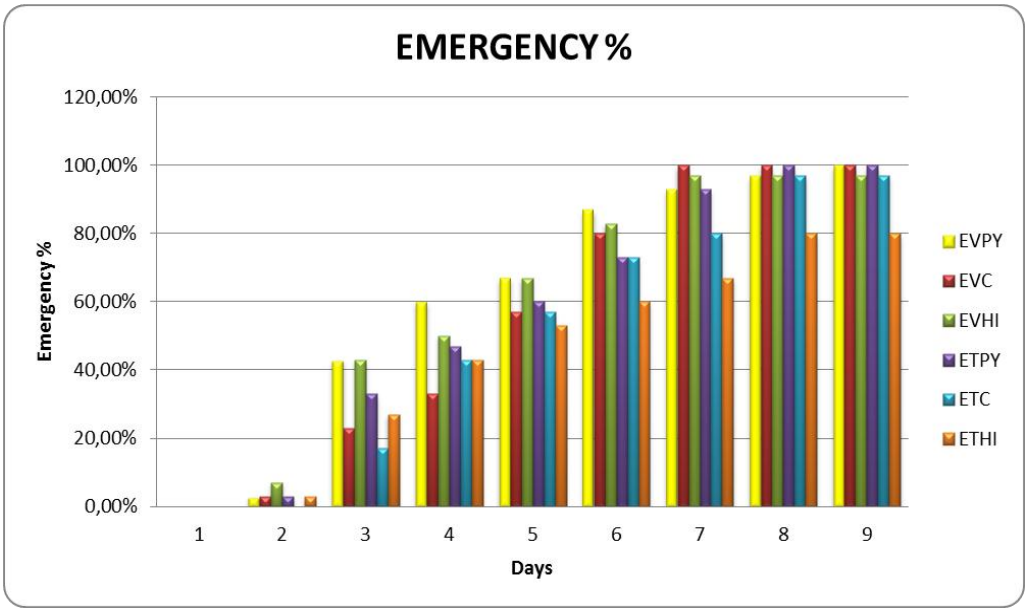


Figure 3. Emergency % Analysis in Petri dishes versus time (days). EVPY(Water irrigation solution A with vermiculite (V) substrate Emergency %) yellow represented, EVC (Water irrigation solution C with vermiculite (V) substrate Emergency %) red represented, EVHI (Water irrigation solution B with vermiculite (V) substrate Emergency %) green represented, ETPY(Water irrigation solution A with peat (T) substrate Emergency %) violet represented, ETC (Water irrigation solution C with peat (T) substrate Emergency %) blue represented and ETHI (Water irrigation solution B with peat (T) substrate Emergency %) orange represented.

Table nº 3: Average of emergency % rate in Petri dishes.

DAY	EVPY	EVC	EVHI	ETPY	ETC	ETHI
1	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
2	3,00%	3,00%	7,00%	3,00%	0,00%	3,00%
3	43,00%	23,00%	43,00%	33,00%	17,00%	27,00%
4	60,00%	33,00%	50,00%	47,00%	43,00%	43,00%
5	67,00%	57,00%	67,00%	60,00%	57,00%	53,00%
6	87,00%	80,00%	83,00%	73,00%	73,00%	60,00%
7	93,00%	100,00%	97,00%	93,00%	80,00%	67,00%
8	97,00%	100,00%	97,00%	100,00%	97,00%	80,00%
9	100,00%	100,00%	97,00%	100,00%	97,00%	80,00%

4.4. Germination rate analysis

For vermiculite substrate (V) no significant variances were found among the irrigation solutions (Figure 4). Nevertheless, a decrease of 4.76% and 9.52 % in germination rate value with irrigation solutions A and B were found in peat substrate (T).

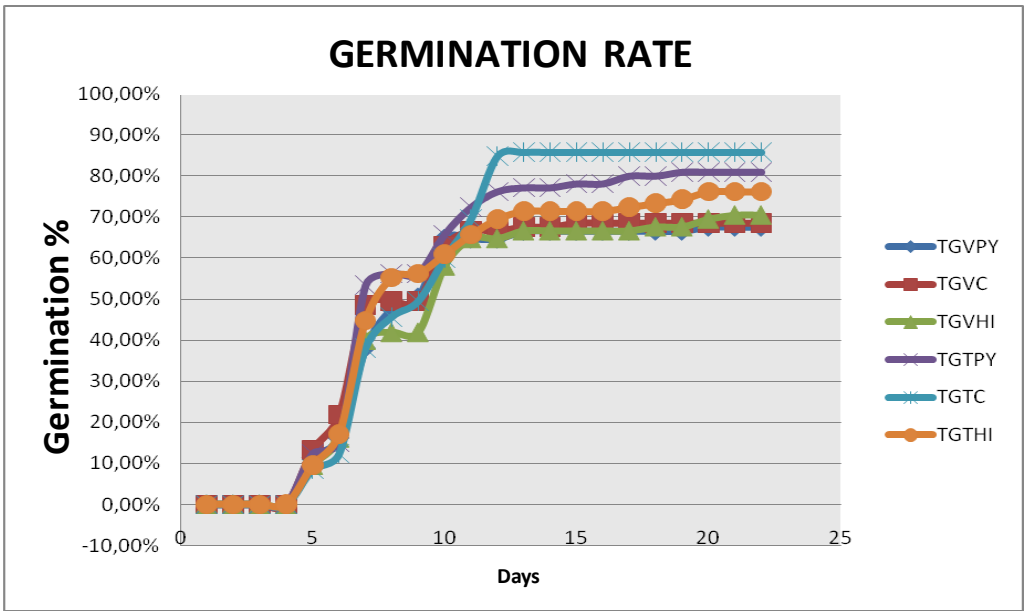


Figure 4. Germination rate versus time (days). TGVPY(Water irrigation solution A with vermiculite (V) substrate Germination rate) dark blue resented, TGVC (Water irrigation solution C with vermiculite (V) substrate Germination rate) red represented, TGVHI (Water irrigation solution B with vermiculite (V) substrate Germination rate) green represented, TGTPY(Water irrigation solution A with peat (T) substrate Germination rate) violet represented, TGTC (Water irrigation solution C with peat (T) substrate Germination rate) blue represented and TGTHI (Water irrigation solution B with peat (T) substrate Germination rate) orange represented.

Table nº 4: Average of the percent of emergency rate.

DAY	TGVPY	TGVC	TGVHI	TGTPY	TGTC	TGTHI
1	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
2	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
3	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
4	0,00%	0,00%	0,00%	0,01%	0,00%	0,03%
5	11,43%	13,33%	9,52%	12,38%	8,57%	9,52%
6	16,19%	21,90%	16,19%	15,24%	12,38%	17,14%
7	38,09%	48,57%	40,00%	53,33%	38,09%	44,76%
8	47,62%	49,52%	41,90%	56,19%	45,71%	55,24%
9	50,47%	49,52%	41,90%	56,19%	49,52%	56,19%
10	64,76%	62,86%	58,10%	65,72%	60,00%	60,95%
11	64,76%	66,67%	64,76%	72,38%	69,52%	65,71%
12	64,76%	66,67%	64,76%	76,19%	84,76%	69,52%
13	66,66%	67,62%	66,67%	77,14%	85,71%	71,43%
14	66,66%	67,62%	66,67%	77,14%	85,71%	71,43%
15	66,66%	68,57%	66,67%	78,10%	85,71%	71,43%
16	66,66%	68,57%	66,67%	78,10%	85,71%	71,43%
17	66,66%	68,57%	66,67%	80,00%	85,71%	72,38%
18	66,66%	68,57%	67,62%	80,00%	85,71%	73,33%
19	66,66%	68,57%	67,62%	80,95%	85,71%	74,28%
20	67,62%	68,57%	69,52%	80,95%	85,71%	76,19%
21	67,62%	68,57%	70,48%	80,95%	85,71%	76,19%
22	67,62%	68,57%	70,48%	80,95%	85,71%	76,19%

4.5. Green leaves rate

For peat substrate (T) a decrease of 8.58% in green leaves rate value with irrigation solution B was found as well as no significant variances were recognized among the irrigation solutions A and C. Referring to green leaves rate should be emphasized that vermiculite substrate had lower value than the obtained in peat substrate regardless of the irrigation water used.

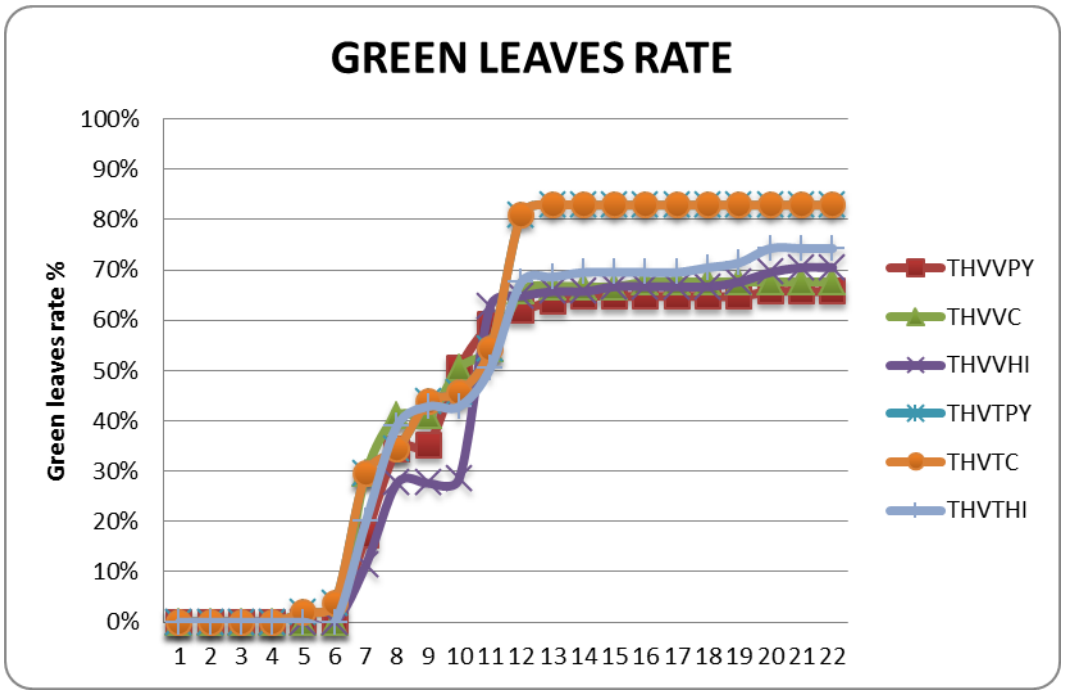


Figure 5. Green leaves rate versus time (days). THVVPY(Water irrigation solution A with vermiculite (V) substrate Green leaves rate) red resented, THVVC (Water irrigation solution C with vermiculite (V) substrate Green leaves rate) green represented, THVVHI (Water irrigation solution B with vermiculite (V) substrate Green leaves rate) violet represented, THVTPY(Water irrigation solution A with peat (T) substrate Green leaves rate) blue represented, THVTC (Water irrigation solution C with peat (T) substrate Green leaves rate) orange represented and THVTHI (Water irrigation solution B with peat (T) substrate Green leaves rate) grey represented.

Table nº5: Average of the percent of green leaves rate.

DAY	THVVPY	THVVC	THVVHI	THVTPY	THVTC	THVTHI
1	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
2	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
3	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
4	0,00%	0,00%	0,00%	0,00%	0,00%	0,00%
5	0,00%	0,00%	0,00%	1,90%	1,90%	0,00%
6	0,00%	0,00%	0,00%	3,81%	3,81%	0,00%
7	17,14%	29,52%	11,43%	29,52%	29,52%	20,00%
8	34,29%	40,95%	27,62%	34,28%	34,28%	39,05%
9	35,24%	40,95%	27,62%	43,81%	43,81%	42,86%
10	50,47%	50,48%	28,57%	45,71%	45,71%	42,86%
11	59,05%	54,29%	62,86%	54,29%	54,29%	50,47%
12	61,90%	65,71%	64,76%	80,95%	80,95%	67,62%
13	63,81%	66,67%	65,71%	82,86%	82,86%	68,57%
14	64,76%	66,67%	65,71%	82,86%	82,86%	69,52%
15	64,76%	66,67%	66,67%	82,86%	82,86%	69,52%
16	64,76%	67,62%	66,67%	82,86%	82,86%	69,52%
17	64,76%	67,62%	66,67%	82,86%	82,86%	69,52%
18	64,76%	67,62%	66,67%	82,86%	82,86%	70,47%
19	64,76%	67,62%	67,62%	82,86%	82,86%	71,43%
20	65,71%	67,62%	69,52%	82,86%	82,86%	74,28%
21	65,71%	67,62%	70,48%	82,86%	82,86%	74,28%
22	65,71%	67,62%	70,48%	82,86%	82,86%	74,28%

4.6. Dry weight analysis:

A decrease on dry weight was present in peat substrate with irrigation water solution B. But in the other hand it appears that an increase in the growth was found using irrigation water solution A and B in vermiculite substrate. Referring to the dry weight rate should be emphasized that the vermiculite substrate has a lower rate than the obtained with peat substrate with the exception of irrigation water solution B.

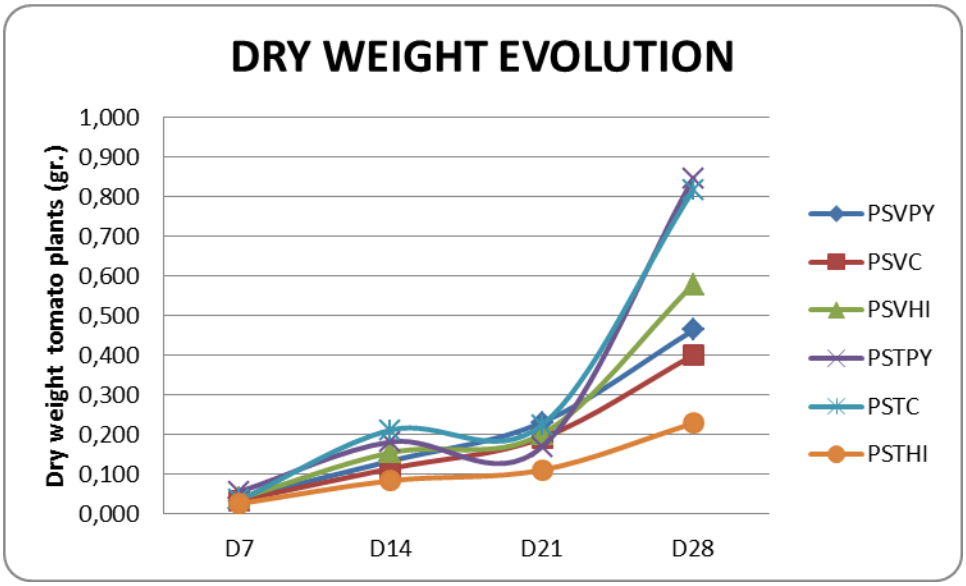


Figure 6. Dry weight versus time (days). PSVPY(Water irrigation solution A with vermiculite (V) substrate Dry weight) dark blue resented, PSVC (Water irrigation solution C with vermiculite (V) substrate Dry weight) red represented, PSVHI (Water irrigation solution B with vermiculite (V) substrate Dry weight) green represented, PSTPY(Water irrigation solution A with peat (T) substrate Dry weight) violet represented, PSTC (Water irrigation solution C with peat (T) substrate Dry weight) blue represented and PSTHI (Water irrigation solution B with peat (T) substrate Dry weight) orange represented.

Table nº6: Average of the percent of dry weight.

DAY	D7	D7	D14	D14	D21	D21	D28	D28
	PSR	PSP	PSR	PSP	PSR	PSP	PSR	PSP
PSVPY	**0,038**	**0,024**	**0,133**	**0,039**	**0,23**	**0,19**	**0,463**	**0,19**
PSVC	**0,033**	**0,022**	**0,114**	**0,037**	**0,119**	**0,141**	**0,4**	**0,141**
PSVHI	**0,038**	**0,018**	**0,154**	**0,039**	**0,201**	**0,473**	**0,579**	**0,473**
PSTPY	**0,055**	**0,016**	**0,181**	**0,025**	**0,169**	**0,48**	**0,846**	**0,48**
PSTC	**0,034**	**0,01**	**0,211**	**0,019**	**0,224**	**0,614**	**0,818**	**0,614**
PSTHI	**0,025**	**0,01**	**0,083**	**0,021**	**0,11**	**0,3**	**0,228**	**0,3**

4.7. Stem growth analysis (length and width)

No significant variances were recognized among the irrigation solutions A-B or C with peat substrate but lower growth trend was found in solution B with vermiculite substrate. Results obtained in peat substrate were higher than in vermiculite, highest values occurring with irrigation water solution A

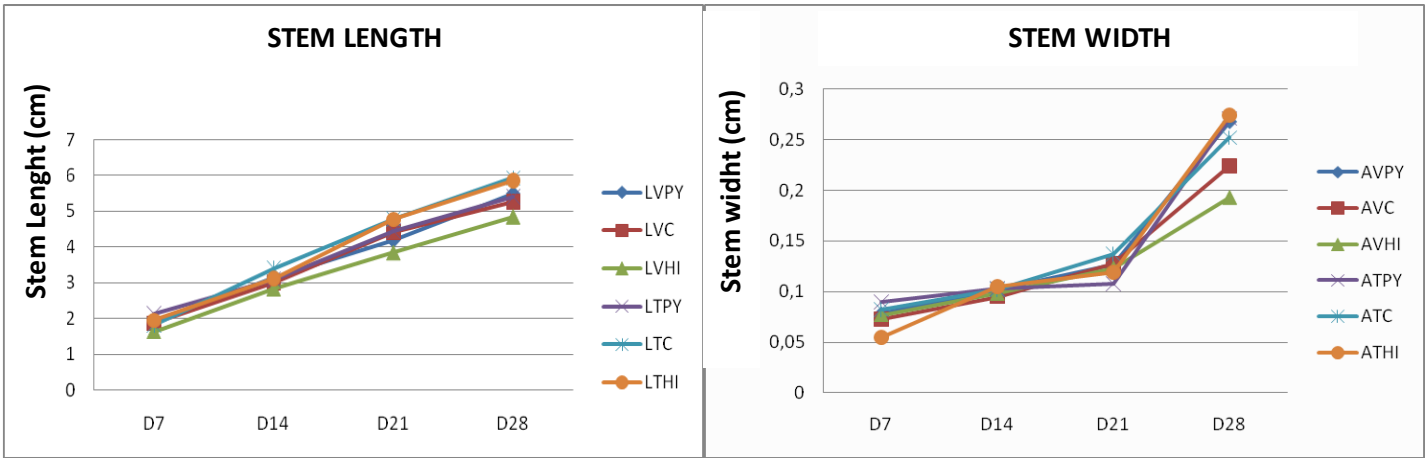


Figure 7.1. Growth stem (width) versus time (days). AVPY(Water irrigation solution A with vermiculite (V) substrate Growth stem (width)) dark blue resented, AVC (Water irrigation solution C with vermiculite (V) substrate Growth stem (width)) red represented, AVHI (Water irrigation solution B with vermiculite (V) substrate Growth stem (width)) green represented, ATPY(Water irrigation solution A with peat (T) substrate Growth stem (width)) violet represented, ATC (Water irrigation solution C with peat (T) substrate Growth stem (width)) blue represented and ATHI (Water irrigation solution B with peat (T) substrate Growth stem (width)) orange represented.

Figure 7.2. Growth stem (length) versus time (days). LVPY(Water irrigation solution A with vermiculite (V) substrate Growth stem (length)) dark blue resented, LVC (Water irrigation solution C with vermiculite (V) substrate Growth stem (length)) red represented, LVHI (Water irrigation solution B with vermiculite (V) substrate Growth stem (length)) green represented, LTPY(Water irrigation solution A with peat (T) substrate Growth stem (length)) violet represented, LTC (Water irrigation solution C with peat (T) substrate Growth stem (length)) blue represented and LTHI (Water irrigation solution B with peat (T) substrate Growth stem (length)) orange represented.

Table nº7: Average of growth stem (length and width)

DAY	D7	D7	D14	D14	D21	D21	D28	D28
	LV	AV	LV	AV	LV	AV	LV	AV
VVPY	1,883	0,078	3,161	0,102	4,193	0,127	5,497	0,268
VVC	1,867	0,073	3,006	0,095	4,411	0,128	5,269	0,224
VVHI	1,633	0,077	2,829	0,098	3,839	0,123	4,846	0,193
VTPY	2,133	0,09	3,083	0,103	4,437	0,108	5,4	0,271
VTC	1,817	0,082	3,394	0,102	4,789	0,137	5,93	0,252
VTHI	1,95	0,055	3,111	0,105	4,773	0,119	5,863	0,274

In order to confirm the previous results variance analysis (ANOVA) were performed. (Massart, 1997) Considering the results obtained, null hypothesis having a calculated F value bigger than the F tabulated (α = 0,02,) was accept, demonstrating that data were significant.

5. DISCUSSION

Microalgae mass production optimization has been investigated in closed and open outdoor systems (Grobbelaar 2007; Sandnes et al. 2005; Zou and Richmond 2000). For commercial objectives, microalgal cultivation is carried out in open, outdoor systems (Huang et al. 2010). The present work shows the possibility of scale Chlorella pyr and Chlorella sp (hy) culture from the laboratory to the greenhouse. Two raceways of 3 m^2 have been successfully used for scale Chlorella pyr and Chlorella sp (hy) cultivation, under greenhouse conditions. Open raceways, however, present several difficulties. High cell densities and efficient control of principal culture parameters had to be achieved, to ensure a sustainable cultivation process (Tredici and Chini Zittelli, 1998). Biomass requires large ground areas and becomes easily contaminated by microalgae and zooplankton (Richmond, 1986) demanding therefore long experience to maintain it successfully. During 90 days both cultures worked in semi-continuous system. Cultures were inoculated on March 2010 with 250 L monoalgal inoculum for each raceway. They were grown at the same conditions and under the same

management. Under temperature and irradiance condition in Gran Canaria Island, from March to May 2010, scale *Chlorella sp (hy)* cultures flocculate and cells fall down the bottom of the raceway at the same time than polysaccharide-like segregation was observed. Cultures were harvesting at the beginning of the stationary phase (1-1.2 optical density) where cultures were nutrients consuming with low growth rate. Semi-continuous system was working during three months, until the culture collapsed, due to the appearance of algal predators and contaminating algal species (Sukenik *et al*., 1993).

For the first phase of germination, seedlings were placed during three days in ITC garage to avoid sunlight. Alveoli (holes) where seeds would be placed later were identified for future monitoring and control. Each of the alveoli seeds were buried in 0.5 - 1 cm depth. After three days of phase 1, the alveoli plates were taken outside outside (greenhouse) to start the emergency phase. Seed daily control was done. Growth phase was carried out simultaneously with the emergency phase. To perform the various parameters analysis random of three seedlings of each treatment were chosen. For each substrates and treatments, seedlings were placed in transparent bags identified with their code, after being removed from the alveoli in a delicate way to prevent breakage. Measurements were carried out immediately after extraction in 7, 14, 21 and 28 day of each trial (Bewley and Blanck, 1982). Three experiments replicated were carried out using representative samples in which irrigation water solution A-B and C was used. Tomato seedling during the different growth phases showed a healthy appearance deprived of visible parasites or diseases.

The plant growth analysis has been developed over the decades as a discipline related to the ecophysiology and agronomy. (Diez, C. et al, 2001). Peat is one of global seedling production substrate more used, its physical, chemical and biological characteristics provide excellent germination and seedling growth, but its high cost and unsustainable exploitation, has begun to restrict it use. This has motivated the search for other alternatives substrates like vermiculite substrate among others (Abad et al. 1998; Budgee, GJ et al. 1989; Harz, K. et al 1986; Peña 2005). Numerous products from organic and mineral as an alternative to organic agriculture are tested in the world. These are foliar applied as stimulators of plant growth and some extent the ground, having effects on soil

biota and physico-chemical properties. (Pohl J., 2002). Substances such as hormones, vitamins and amino acids had been studied in bacteria during the decomposition of organic matter (Coyne M.; 2000). Microorganisms and lichen-like were found in our soils after 10 day of growth, without affecting the growth of plants, compounds might be part of the bio-stimulant effect. The presence of microalgae culture water irrigation favors soil fertility, improves organic matter and makes phosphorus available for plants and edaphic medium. The effects achieved by *Chlorella pyr and Chlorella hy culture as source of irrigation water*, corroborate the points made by scientists who have devoted serious efforts to related to the synthesis, biological activity and practical applications of a new class of plant growth regulators (Laugart and Romero (2003), Casanova et al., 2003).

Germination rate is an important factor related with agriculture industrial production because of the significance uniformity of plans for bidding (Bridges et al. 1997). No substantial differences were attained in germination test with V substrate although the results achieved, showed a 10% decrement in relation to substrate T. Uniformity was observed in seedlings as a favorable indicator about the future growth of tomato plants. At that point, it's important to underline that germination rate results in peat substrate were more efficient than vermiculite ones. These marks prompts us to say that peat has best physical, chemical and nutrition conditions which promotes germination rate and confirm peat substrate capacity of moisture retention during microbiological processes, temperature control, pH, etc. Similar data were obtained by Jacob et al. 1993. With an average of 80.90 % and 68.89%, substrate T and V respectively, seedlings presented two fully deployed cotyledon leaves, which designate their level of development, indicative of speed and uniformity seed germination (Fernandez-Bravo, C. et al. 2000). No considerable differences were attained in green leaves results between irrigation water solution A and C even though the marks achieved, showed a 8.58% decrement in relation to solution B. Higher performance in solution A versus B was demonstrated in this part of the research. Vermiculite values obtained compared to peat ones were consistent with the expected trend. Tomato stem thickness has a high importance related to the post production (Casanova et al. 2006). Results obtained in peat substrate were higher than in vermiculite, highest values occurring with irrigation water

solution A. Our results disagree with the results obtained by Gómez et al 1997, Terry et al. 1997 and Sander et al 2006, where there was a general homogeneous behavior for stem diameter in all treatments in tomatoes plants.

The results obtained for several indicators confirm a bio-stimulant effect of microalgae irrigation water in tomato crop. *Chlorella pyrenoidosa* irrigation water show average values comparable to control treatment even in some variables higher. On the other hand marks obtained by *Chlorella sp* (hydroponic water isolated) not as good as those of *Chlorella pyrenoidosa* remain positive. *Solanum lycopersicum var. charony* crops can be enhanced by appropriate utilization of microalgae culture. Mechanisms of action of this complex bio-stimulating effect will only be revealed through the use of techniques developed for molecular biology, metabolomics, and genomics. The evidence establishes that benefits derived from the use of irrigation water from *Chlorella pyrenoidosa* and *Chlorella sp* (hydroponic water isolated) can be undeniable in agriculture. But further studies are needed to encompass the entire life cycle of the tomato production.

6. CONCLUSIONS

1. The results obtained in this work show that use microalgae culture *Chlorella pryrenoidosa and Chlorella sp* (hydroponic water isolate) growth media as irrigation water resulted in no changes in the seedlings studied, nor in its biological activity, as well as environmental conditions where these are developed.

2. Flocculation and polysaccharide-like segregation observed in chlorella sp (hydroponic water isolated) should be studied in depth to ascertain whether these polysaccharides could be the cause of the decrease of the values compared with the results of *Chlorella pyrenoidosa.*

3. Even if vermiculite substrate achieved lower values efficiency than peat substrate, these results remain positive. Therefore, the use of irrigation water from *Chlorella pyrenoidosa* and *Chlorella sp* (hydroponic water isolated) culture could favor the production of *Solanum lycopersicum charony* crops with special attention in poor soils. This can be of great importance in relation to a lower environmental impact and a cost-effective tomato crop production.

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