Effectiveness of Microalgae-Based Biostimulant STIMUTER® on Agronomic and Quality Traits in Tomato (*Solanum lycopersicum L.*)

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INTRODUCTION

The root system is essential for plant growth, development, and survival. Besides anchoring plants to soil, it enables water and nutrient uptake and phytohormone synthesis. Root morphology, encompassing density, length, absorptive surface area, and branching, is influenced by environmental conditions and resource availability but can also be modulated through agronomic practices or biostimulants [1]. Interest in sustainable strategies to enhance root performance has grown, driven by the need to improve crop productivity. Stimulating root growth improves resource-use efficiency and tolerance to abiotic stress [2]. Among explored approaches, algae have emerged as promising tools to promote root and stress resilience. Algae are aquatic photosynthetic organisms with wide morphological, physiological, and phylogenetic diversity, including unicellular and multicellular forms, ranging from prokaryotic cyanobacteria to eukaryotic green, brown, and red algae [3]. Algae are classified into macroalgae and microalgae, differing in cellular structure, size, and habitat [4]. Macroalgae are multicellular and typically inhabit marine environments. They are rich in complex polysaccharides like agar, carrageenan, and alginates, but large-scale cultivation is limited by environmental factors [5]. Microalgae, in contrast, are unicellular or colonial microorganisms that grow in suspension in natural or artificial aquatic environments. This group includes chlorophytes (e.g., *Chlorella* spp.), diatoms, cyanobacteria, and others [5,6]. This study focuses on microalgae—specifically *Chlorella vulgaris*—as a source of bioactive compounds for sustainable agriculture, with emphasis on root growth–promoting effects [6]. *C. vulgaris* is a unicellular green microalga (Chlorophyta) known for its physiological versatility and biotechnological potential. Its rapid growth, high photosynthetic efficiency, and ability to accumulate bioactive metabolites make it ideal for applications in nutraceuticals, medicine, agriculture, and bioenergy [7]. Morphologically, *C. vulgaris* consists of small (2–10 µm) spherical cells with a robust cell wall and a chloroplast containing chlorophylls a and b [7,8]. Biochemically, *C. vulgaris* is rich in proteins (up to 60% dry weight) with a balanced amino acid profile, lipids, carbohydrates, pigments (chlorophylls, carotenoids like lutein), vitamins, and minerals [7,8]. STIMUTER® is a liquid product containing *C. vulgaris* and plant extracts, originally developed by Pireco and formulated by Diachem S.p.A., suitable for foliar application and fertigation. The proposed trial, commissioned by Diachem S.p.A. to the CRO Res Agraria S.r.l. to register STIMUTER® as a CE biostimulant according to regulation (EU) 2019/1009 of 5 June 2019, has shown positive effects on root development and plant physiology.

MATERIALS AND METHODS

This study on tomato (*Solanum lycopersicum L.*) was set up in Tortoreto Lido (Abruzzo Region, Italy) in a growth chamber. The variety selected for this trial was Roma VF, widely spread as processing variety. The experimental design consisted of one complete randomized block per treatment, arranged with four replicates per treatment and five plants were set up in each experimental unit according to CEN General principles guideline [9].
The plants were transplanted by hand in peat substrate in small pots (72 cm² area) with a row spacing of 12 cm and spacing within row of 12 cm. Controlled conditions were maintained by adjusting humidity, temperature and photoperiod throughout the trial: 16 hours of light per day, a minimum temperature of 22°C (for 8 hours per day), a maximum temperature of 25°C (for 16 hours per day). Relative humidity was set between a minimum of 60% and a maximum of 75%.
The test item is STIMUTER® a biostimulant aimed at the vegetative increase of the root system in compliance with CEN Quality traits guideline [10]. STIMUTER® was provided by the company Diachem S.p.A. (Caravaggio, Italy) and was tested with two different application modes to cover different farmer practices. The dose rate applied was 10 L/ha and 20 L/ha by foliar spray and 10 L/ha and 20 L/ha by drip irrigation. An untreated control was included in the protocol as well to prove the efficacy of the test item.
Throughout the trial period, the product was applied 4 times with a water volume of 500 L/ha for foliar spraying and 10.000 L/ha in drip irrigation. The first application was done at BBCH 13 (3rd leaf on main shoot unfolded) [11], whereas the following applications every 7 days as recommended by the manufacturer.
Non-destructive assessments took place at 7 DAA, 7 DAB, 7 DAC and 7 DAD to monitor the early crop stages by measuring plant height (cm), SPAD index (chlorophyll content) and NDVI were measured.
At 7 DAC and 7 DAD the plants were cut, the epigeal part and the hypogeal part of the plant were weighed to determine the root's fresh and dry biomass (g) and root length (cm).
Data were analysed by means of analysis of variance (ANOVA) using software ARM [12] .
Results obtained were indicated by a letter, and treatment means with no letters in common are significantly different.

TABLE 1: Trial Protocol

TRT N°	TYPE	TREATMENT NAME	RATE	RATE UNIT	APPL TIMING	APPL CODE	APPL DESCRIPTION	APPL AMOUNT	APPL UNIT
1	CHK	**Untreated Check**	-	-	-	-	-	-	-
2	BIOSTIM	**STIMUTER**	10	L/ha	A: 3 days after transplanting, then every 7 days	ABCD	Foliar spray	500	L/ha
3	BIOSTIM	**STIMUTER**	20	L/ha	A: 3 days after transplanting, then every 7 days	ABCD	Foliar spray	500	L/ha
4	BIOSTIM	**STIMUTER**	10	L/ha	A: 3 days after transplanting, then every 7 days	ABCD	Drip irrigation	10.000	L/ha
5	BIOSTIM	**STIMUTER**	20	L/ha	A: 3 days after transplanting, then every 7 days	ABCD	Drip irrigation	10.000	L/ha

RESULTS AND CONCLUSIONS

PLANT HEIGHT

Plant height is an important agronomical trait especially in the first phenological stage of tomato plant development. Under standard conditions, plant height is directly related to the development of plant biomass. At every assessment, plant height in plants treated with STIMUTER® (all treatments) statistically overpowered the untreated check. Especially after four application (7 DAD) an increase of +9.8% and +13.4% compared to the untreated check control was recorded in the theses applied in spray respectively treated with STIMUTER® at 10 L/ha and 20 L/ha. Regarding the theses applied in fertigation, an increase of +13.10% and +10.80% compared to the untreated check control was detected for the theses respectively treated with STIMUTER® at 10 L/ha and 20 L/ha.

ROOT LENGTH

Root biostimulation is the primary focus of investigation for this product. The trial was conducted in growth chamber conditions to detect the effect of STIMUTER® on root growth. Root growth is measured using various methods, including root length. The product, applied by spray at 10 L/ha, increased root length by +50.3% compared to the untreated control and by +74.9% when applied at 20 L/ha. Both spray treatments are statistically different compared to the untreated control. The treatments with drip applications are also statistically different compared to the untreated check control, with higher values than both the control and the spray treatments. Indeed, when the product was applied at 10 L/ha, an increase of +131.1% was observed compared to the untreated control, and when the product was applied at a dose rate of 20 L/ha, an increase of +123.5% was observed compared to the untreated control.

ROOT WEIGHT

Another morphological parameter assessed on root system was the root biomass (fresh weight and dry matter content). Regarding dry matter content, the following increases can be observed compared to the control: spray at 10 L/ha +92.7% and at 20 L/ha +119.4%, drip at 10 L/ha +133.9% and at 20 L/ha +153.9%. The effects on root weight are statistically more evident compared to the untreated check in plants treated with the drip application method, and this could be due to the direct contact between the root and the product applied in fertigation.

CHLOROPYLL CONTENT- NDVI

Multispectral assessments such as NDVI and SPAD were done to evaluate the effect of STIMUTER® on chlorophyll content. Chlorophyll content is influenced by the product: after 4 applications (7 DAD) all the treated treatments had statistically higher values of SPAD Index and NDVI index.

The results obtained revealed that application of STIMUTER®, at the concentration used in this trial, improved the quality traits of tomato plants, increasing plant height, root length and root biomass. In addition, the biostimulant treatment significantly increased the chlorophyll content (SPAD index) and NDVI.
The best dosage for foliar application of the product test seems to be between 10 and 20 L/ha and for drip irrigation between 10 and 20 L/ha. Moreover, no phytotoxic symptoms were observed in plots treated with the test products and no unexpected effects were observed on the crop from any of the treatments.
The trial is part of the registration process to make STIMUTER® a CE biostimulant. Following a positive evaluation by EFCI [13] and the subsequent issuance of the CE mark (registration number: DE.4791), the efficacy has been confirmed through scientifically valid data.

FIGURE 1: Plant height 7 DAD - STIMUTER®

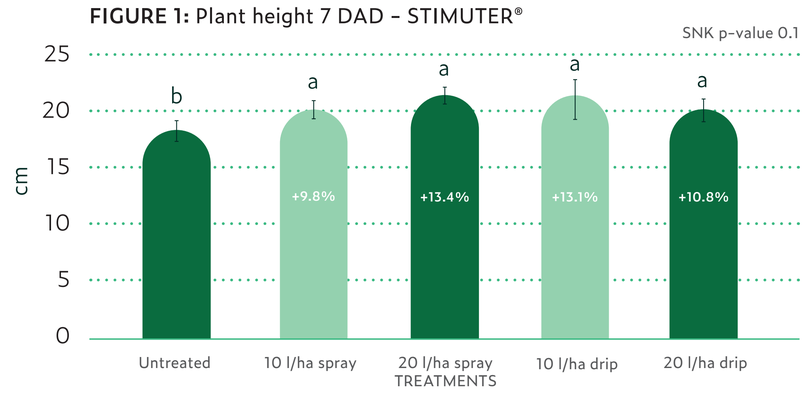

FIGURE 2: Root length 7 DAD - STIMUTER®

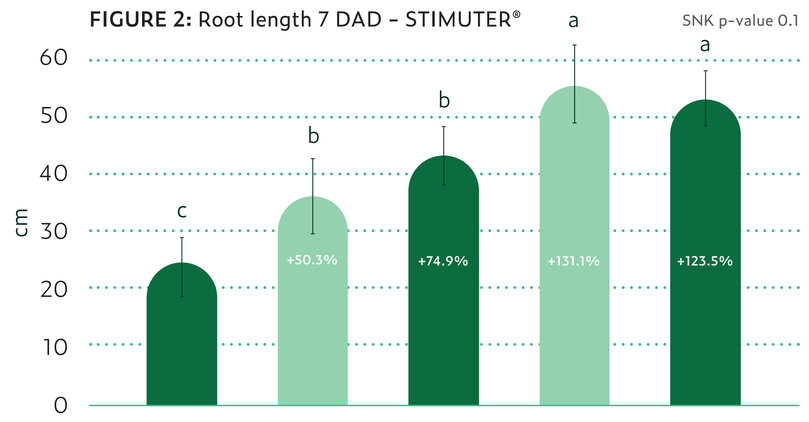

FIGURE 3: Root weight 7 DAD - STIMUTER®

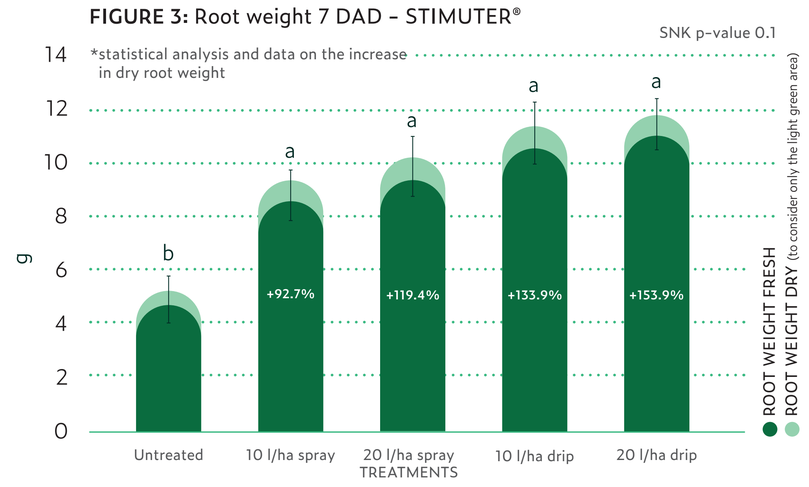

FIGURE 4: Samples harvest for the test.

On the right the treated samples, on the left the control.

TABLE 2:
Chlorophyll content related index 7 DAD - STIMUTER®

TREATMENTS	NDVI (0-1) INDEX		SPAD (0-99) INDEX	
Untreated	0,71±0,02	(b)	37,2±0,94	(b)
10 L/ha spray	0,77±0,01	(a)	39,02±1,38	(a)
20 L/ha spray	0,79±0,04	(a)	39,42±0,60	(a)
10 L/ha drip	0,83±0,04	(a)	41,19±1,43	(a)
20 L/ha drip	0,78±0,02	(a)	40,37±1,58	(a)

(Value ± standard deviation)

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