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One Plant-Based Biostimulant Stimulates Good Performances of Tomato Plants Grown in Open Field †

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Abstract: Most agricultural practices have evolved towards biological and sustainable systems. The purpose of modern agriculture is to reduce inputs without reducing yield and quality. This objective can be achieved through breeding programs and the identification of organic molecules capable of activating plant metabolism. Biostimulants contain a wide range of mostly still unknown bioactive compounds. These products are generally able to improve the plant's nutrient utilization efficiency and increase tolerance to biotic and abiotic stresses. The aim of this study was to determine biometric measurements and metabolic profiling of two tomato genotypes grown in open field and treated or not with a plant-derived biostimulant named CycoFlow (Agriges). The application of the biostimulant stimulated growth (plants up to 55.06% higher) and yield per plant (up to 111.66%). In plants treated with the biostimulant, antioxidants and pigments contents in fruit were higher compared to non-treated plants. In particular, the content of β-carotene increased after treatments with CycoFlow. The present study proves that the application of plant-derived biostimulant can increase tomato performance in the field.

Keywords: sustainable agriculture; bioassay; crop; *Solanum lycopersicum*

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1. Introduction

There are many works in literature aimed at finding alternative management practices able to improve the growth, productivity and quality of crops and that are also environmentally friendly [1]. For these reasons, in modern agriculture the use of biostimulants is increasingly becoming an interesting and widespread option [2]. According to Traon [3] "A biostimulant is any substance or microorganism, in the form in which it is supplied to the user, applied to plants, seeds or the root environment with the intention of stimulating the natural processes of plants for the benefit of efficiency, the use of nutrients and/or tolerance to abiotic stress, regardless of its nutrient content, or any combination of such substances and/or microorganisms intended for this use". In the wide range of biostimulants, particular attention has been given to compounds of plant origin whose effects is linked to their involvement in the metabolism, signaling and hormonal regulation of growth and development of the plant [1,4–7]. Tomato (*Solanum lycopersicum* L.) is one of the most consumed vegetables worldwide also owing to the development of products such as soups, juices, purees, and sauces [8]. Tomato is an essential component of the Mediterranean diet and of other traditional diets. Given the key role of this crop, research aimed at improving tomato quality could contribute to global food production. To verify this hypothesis, we used a plant-based biostimulant named CycoFlow (Agriges) and we performed biometric measurements and biochemical analyses on two different tomato genotypes grown in open field and treated or not with this novel plant-based biostimulant.

2. Methods

Experiments were carried out at an agronomy farm located in Apollosa, (Benevento), Italy (latitude 41°5′42″36 N; longitude 14°42′22″32 E) on a clay-loam soil. Four weeks following seeding, after the third true leaf was fully expanded, tomato plants (genotype E42, available at the University of Naples, Department of Agricultural Sciences and LA3120, Tomato Genetics Resource Centre, TGRC, University of California, CA, USA) were transplanted into open field in May 2020. Tomato plants were grown following the standard agronomical practices. The experimental design consisted of a completely randomized design with three replicates per treatment and ten plant per each biological replication. There were two different groups: one control, which did not receive any biostimulant, and one that was treated with the biostimulant. The biostimulant was applied at the moment of transplanting and thereafter every 15 days, until the end of the cultivation cycle for a total of four applications, by fertigation with a 3 g per liter solution. CycoFlow is a plant extracts-based biostimulant produced by the Agriges company (Benevento, Italy), which is rich in glutamic acid (including glutamine) and glycine betaine, peptides, nucleotides, vitamins B, trace elements and other growth factors. Its chemical composition contains total nitrogen of 4.5% and organic carbon of 19.5%. The biostimulant has a pH of 5.0, a density of 1200 kg/m^3 and an EC value of 15 dS/m [9]. Pollen viability was analyzed using five flowers per plant sampled from three different plants per replicate with DAB test according to Dafni et al. [10]. Harvesting started at the beginning of August 2020. Six plants per treatment were collected for biomass determination. Shoot biomass was calculated as the sum of aerial vegetative plant parts (leaves + stems) and fruits were counted and weighted. Plant material was put in a stove at 85 °C for 24 h and dry weight of shoot was measured. Samples of freshly harvested fully ripened fruits were collected from each plot to determine antioxidant and pigments content by a colorimetric assay on freeze dried and finely ground sub-samples. The evaluation of total carotenoids, lycopene and β-carotene was carried out according to the method reported by Wellburn and by Zouari et al., as modified by Rigano et al. [11–13]. Measurements of the content of reduced ascorbic acid (AsA) was carried out by using a colorimetric method [14], with modifications reported by Rigano et al. [15,16]. Total phenolic compounds were evaluated by using the Folin–Ciocalteu assay with modifications reported by Rigano et al. [15]. Hydrophilic antioxidant activity (HAA) determination was carried out according to the 2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method [17]. Data were analyzed by ANOVA and means were compared by the Tukey's test.

3. Results and Discussion

As reported in Figure 1, the application of a plant-based biostimulant named CycoFlow (Agriges) resulted in higher height and higher fresh and dry biomass of the vegetative plant parts only in the genotype E42 compared to non-treated plants. Marketable yield and its component, fruit number, were significantly affected by biostimulant treatment according to ANOVA analyses (Table 1). On the contrary, CycoFlow application had no effect on pollen viability (Table 1). In both genotypes the application of CycoFlow resulted in significantly higher yields compared to non-treated control plants (+111.67% in E42 and +43.37% in LA3120). The observed effect may be due to the physiological mechanisms triggered in tomato plants after biostimulant application and linked to an increased content of signaling molecules, which are the main components of this biostimulant of plant origin [9]. Accordingly, it has been reported that plant growth, fruit set and yield can be improved by the cytokinin-like activity of the biostimulant applied [9]. CycoFlow application likely increased plant development and yield by stimulating cell proliferation by signaling molecules, such as specific amino acids linked to nitrogen metabolism (i.e., glutamic and aspartic acids) and soluble peptides.

Table 1. Analyses of variance for all measurements in fruit of two tomato genotypes treated with the biostimulant CycoFlow.

	Significance		
	G	**B**	**GxB**
Height (cm)	***	***	***
Pollen viability	**	ns	ns
Shoot FW (g)	**	**	*
Shoot DW (g)	ns	*	*
N° fruits	***	***	*
Yield (kg/pt)	ns	***	ns
Ascorbic acid (mg/100 g FW)	***	***	***
Carotenoids (mg/100 g FW)	***	ns	**
β-carotene (mg/100 g FW)	***	**	***
Lycopene (mg/100 g FW)	***	ns	ns
Phenols (mg/100 g FW)	***	***	ns
HAA Abts (μmol TE/100 g FW)	***	***	***

G = genotype, B = biostimulant; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.

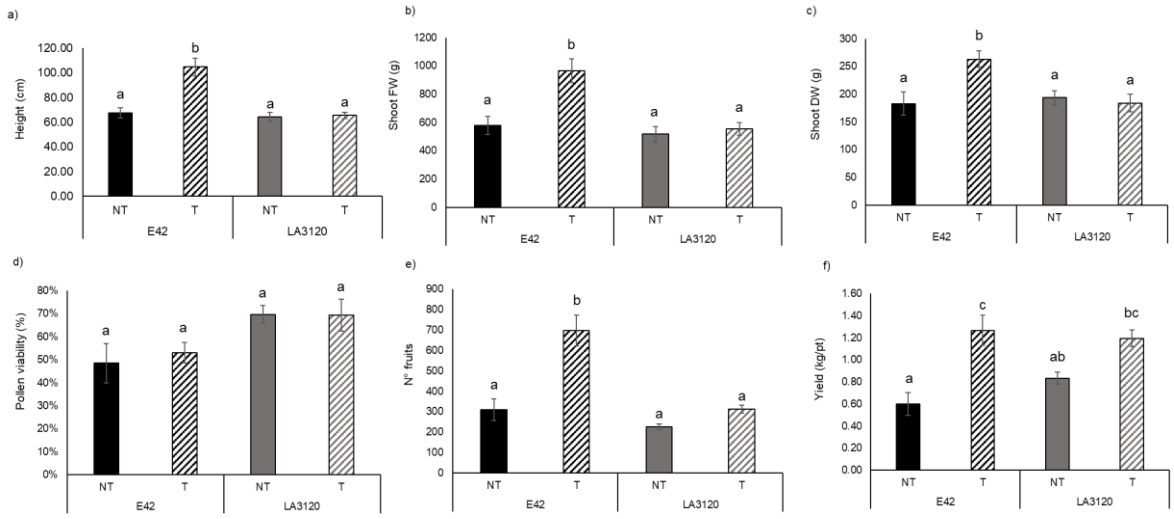

Figure 1. Effect of CycoFlow on (**a**) height, (**b**) shoot fresh weight, (**c**) shoot dry weight, (**d**) pollen viability, (**e**) number of fruits (**f**) yield per plant on two tomato genotypes. Values are mean ± SE. Different letters indicate significant differences based on Tukey's test ($p \leq 0.05$).

Fruit vegetables, and in particular tomato, are considered good sources of antioxidant molecules such as lycopene, ascorbic acid and polyphenols. The influence of biostimulant application on antioxidant activities and bioactive compounds is reported in Figure 2. The treatment with the biostimulant increased the content of ascorbic acid only in the genotype E42 (Figure 2a). The content of ascorbic acid increased by 28.59% in fruit from E42 treated plants, while it decreased by 14.36% in fruit from LA3120 treated compared to non-treated plants (Figure 2a). Only in the LA3120 treated plants there was a significant decrease in phenol content equal to 17.47% (Figure 2b). Moreover, a significantly higher antioxidant activity HAA was demonstrated in fruits from E42 plants treated with CycoFlow (Figure 2c). These results are in agreement with results previously obtained in soybean seeds, even if the reported effects depended on the kind of biostimulant applied and on the number of applications [18]. According to ANOVA analyses the application of biostimulant did not have a significant effect on either the carotenoid or the lycopene content (Table 1). Similar results were obtained by Chehade et al. [19] in tomato. On the contrary, Rouphael et al. [20] demonstrated that in tomato foliar applications of a legume-

derived protein hydrolysate had an effect also on lycopene content. On the other hand, the β-carotene content is not influenced by the application of the biostimulant in the LA3120 genotype, but in the E42 treated plants there was an increase in the β-carotene content as shown in Figure 2e.

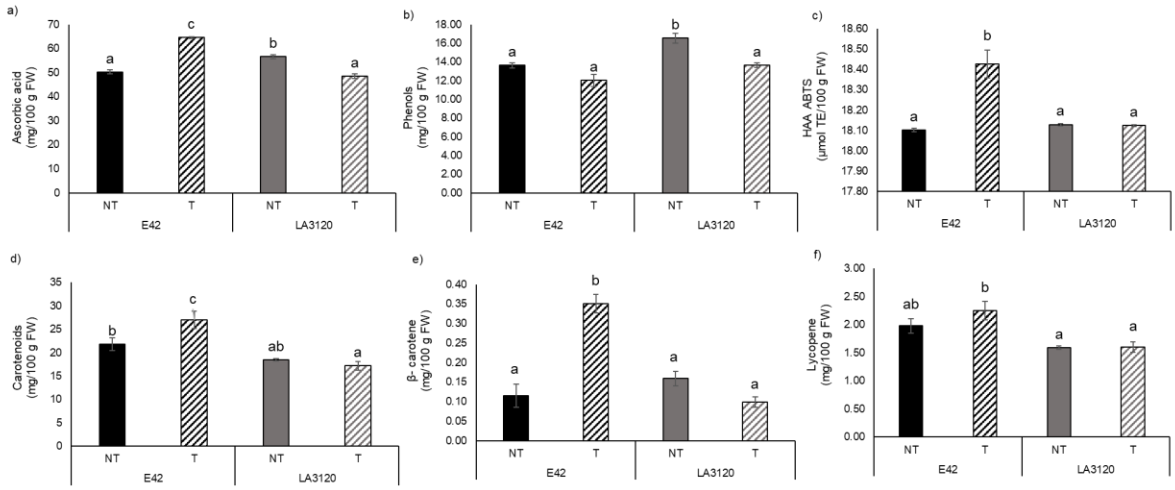

Figure 2. Effect of CycoFlow on the content of (**a**) ascorbic acid, (**b**) phenols, (**c**) hydrophilic antioxidant activities (HAA), (**d**) carotenoids (**e**) β-carotene (**f**) lycopene in fruit of two tomato genotypes. Values are mean ± SE. Different letters indicate significant differences based on Tukey's test ($p \leq 0.05$).

4. Conclusions

From the research conducted on the two tomato genotypes, the effects of the application of a biostimulant based on plant extracts on fruit yield, nutritional and functional attributes emerged. Controversial results have arisen from the comparison between the two genotypes, since the effect of the biostimulant appears to be clearer only in the genotype E42. Altogether, the present study highlighted that the application of biostimulants may contribute to make sustainable a conventional tomato cultivation system.

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References

1. Parađiković, N.; Teklić, T.; Zeljković, S.; Lisjak, M.; Špoljarević, M. Biostimulants research in some horticultural plant species. A review. *Food Energy Secur.* **2019**, *8*, e00162, doi:10.1002/fes3.162.
2. Pascual, J.A.; Ceglie, F.; Tuzel, Y.; Koller, M.; Koren, A.; Hitchings, R.; Tittarelli, F. Organic substrate for transplant production in organic nurseries. A review. *Agron. Sustain. Dev.* **2018**, *38*, 35, doi:10.1007/s13593-018-0508-4.
3. Traon, D.; Laurence, A.; Ferdinand, Z.; Du Jardin, P. A Legal Framework for Plant Biostimulants and Agronomic Fertilizer Additives in the EU. Report for the European Commission, Enterprise & Industry Directorate—General. Contract n° 255/PP/ENT/IMA/13/1112420. 2014. Available online: http://publications.europa.eu/resource/cellar/dbeffd43-98a5-4e39-a930-7dfa21816f8c.0001.02/DOC_1 (accessed on).
4. Calvo, P.; Nelson, L.; Kloepper, J.W. Agricultural uses of plant biostimulants. *Plant Soil* **2014**, *383*, 3–41, doi:10.1007/s11104-014-2131-8.

5. Canellas, L.P.; Olivares, F.L.; Aguiar, N.O.; Jones, D.L.; Nebbioso, A.; Mazzei, P.; Picollo, A. Humic and fulvic acids as biostimulants in horticulture. *Sci. Hortic.* **2015**, *196*, 15–17, doi:10.1016/j.scienta.2015.09.013.
6. Nardi, S.; Pizzeghello, D.; Schiavon, M.; Ertani, A. Plant biostimulants: Physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism. *Sci. Agric.* **2016**, *73*, 18–23, doi:10.1590/0103-9016-2015-0006.
7. Shah, Z.H.; Rehman, H.M.; Akhtar, T.; Alsamadany, H.; Hamooh, B.T.; Mujtaba, T.; Chung, G. Humic substances: Determining potential molecular regulatory processes in plants. *Front. Plant Sci*. **2018**, *9*, 1–12, doi:10.3389/fpls.2018.00263.
8. Del Giudice, R.; Petruk, G.; Raiola, A.; Barone, A.; Monti, D.M.; Rigano, M.M. Carotenoids in Fresh and Processed Tomato (*Solanum lycopersicum*) Fruits Protect Cells from Oxidative Stress Injury. *J. Sci. Food Agric.* **2016**, *97*, 1616–1623.
9. Francesca, S.; Arena, C.; Hay Mele, B.; Schettini, C.; Ambrosino, P.; Barone, A.; & Rigano, M.M. The use of a plant-based biostimulant improves plant performances and fruit quality in tomato plants grown at elevated temperatures. *Agronomy* **2020**, *10*, 363, doi.org/10.3390/agronomy10030363.
10. Dafni, A. *Pollination Ecology: A Practical Approach*; Oxford University Press: Oxford, UK, 1992; p. xiv+250.
11. Wellburn, A.R. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J. Plant Physiol*. **1994**, *144*, 307–313.
12. Zouari, I.; Salvioli, A.; Chialva, M.; Novero, M.; Miozzi, L.; Tenore, G.C.; Bagnaresi, P.; Bonfante, P. From root to fruit: RNA-Seq analysis shows that arbuscular mycorrhizal symbiosis may affect tomato fruit metabolism. *BMC Genom.* **2014**, *15*, 221.
13. Rigano, M.M.; Arena, C.; Di Matteo, A.; Sellitto, S.; Frusciante, L.; Barone, A. Eco-physiological response to water stress of drought-tolerant and drought-sensitive tomato genotypes. *Plant Biosyst.* **2016**, *150*, 682–691.
14. Stevens, R.; Buret, M.; Garchery, C.; Carretero, Y.; Causse, M. Technique for rapid small-scale analysis of vitamin C levels in fruit and application to a tomato mutant collection. *J. Agric. Food Chem.* **2006**, *54*, 6159–6165.
15. Rigano, M.M.; Raiola, A.; Tenore, G.C.; Monti, D.M.; Del Giudice, R.; Frusciante, L.; Barone, A. Quantitative trait loci pyramiding can improve the nutritional potential of tomato (*Solanum lycopersicum*) fruits. *J. Agric. Food Chem.* **2014**, *62*, 11519–11527.
16. Rigano, M.M.; Lionetti, V.; Raiola, A.; Bellincampi, D.; Barone, A. Pectic enzymes as potential enhancers of ascorbic acid production through the d-galacturonate pathway in Solanaceae. *Plant Sci.* **2018**, *266*, 55–63.
17. Miller, J.N.; Rice-Evans, C.A. Factors influencing the antioxidant activity determined by the ABTS$^{\bullet+}$ radical cation assay. *Free Radic. Res.* **1997**, *26*, 195–199.
18. Kocira, S.; Szparaga, A.; Kocira, A.; Czerwinska, E.; Wojtowicz, A.; Bronowicka-Mielniczuk, U.; Koszel, M.; Findura, P. Modeling biometric traits, yield and nutritional and antioxidant properties of seeds of three soybean cultivars through the application of biostimulant containing seaweed and amino acids. *Front. Plant Sci.* **2018**, *9*, 388.
19. Chehade, L.A.; Al Chami, Z.; De Pascali, S.A.; Cavoski, I.; Fanizzi, F.P. Biostimulants from food processing by-poducts: Agronomic, quality and metabolic impacts on organic tomato (*Solanum lycopersicum* L.). *J. Sci. Food Agric*. **2017**, *98*, 1426–1436.
20. Rouphael, Y.; Colla, G.; Giordano, M.; El-Nakhel, C.; Kyriacou, M.C.; De Pascale, S. Foliar applications of a legume-derived protein hydrolysate elicit dose-dependent increases of growth, leaf mineral composition, yield and fruit quality in two greenhouse tomato cultivars. *Sci. Hortic.* **2017**, *226*, 353–360.