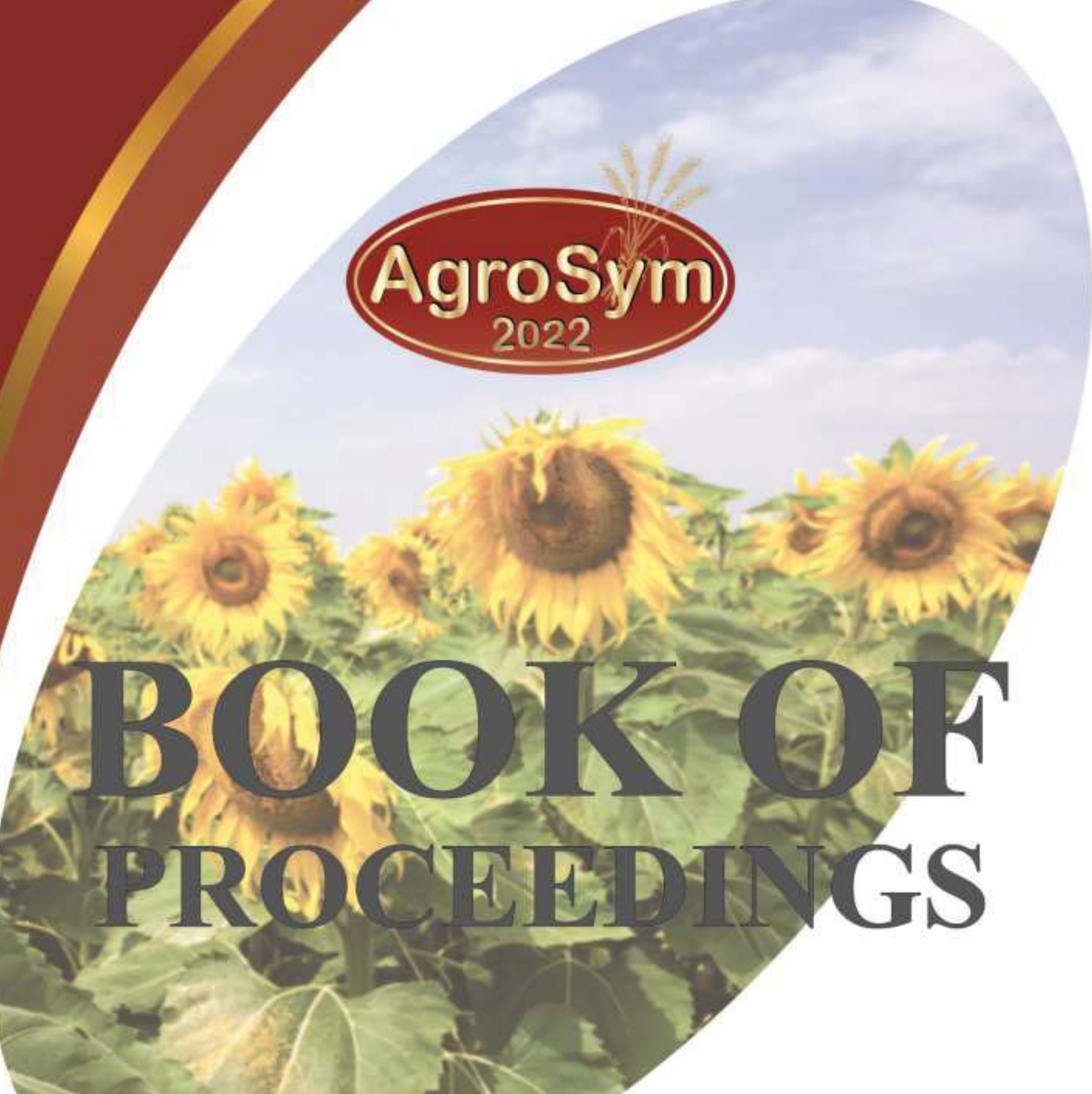

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EFFECTS OF BIOSTIMULATORS ON GROWTH OF TOMATO PLANTS CULTIVATED UNDER PROTECTED AND FIELD CONDITIONS

Slavica DUDAŠ[1], Markos BULIĆ[1], Martina PERSIĆ[1], Michael H. BÖHME*[2]

[1]Polytechnic of Rijeka, Agronomy department in Poreč, Croatia
[2]Humboldt-Universität zu Berlin, Faculty of Life Sciences, Dept. Horticultural Plant Systems, Germany
*Corresponding author: michael.boehme@hu-berlin.de

Abstract

Biostimulators can be used in order to stabilise plant growth in even unfavourable climate conditions or fluctuating conditions in the rhizosphere and they stimulate the process of the formation of plant-organs making the plants resistant to diseases and viruses. In our investigations with tomato plants, liquid substances based on different organic compounds such as lignite coal (Humates), waste of food production (Lactate), algeas (Megafol), plant-based substances (Čudomiks,) and microorganisms such as *Bacillus subtilis* were used as biostimulators. The effects of biostimulators were investigated in tomato soilless cultivation, cv. Ferrari RZ, in the greenhouse of the experimental station in Berlin. Humates, lactates, and *B. subtilis* were added to the nutrient solution, single or mixed and well sprayed on the leaves. Humates improved plant growth even when the EC was very high. In the case of extreme pH values of 4.5 or 7.5 better growth parameters were recorded when lactate had been added to the nutrient solution. In some cases, the development of tomato plants was also enhanced by combinations of humates, lactates, and B. subtilis. The field experiment was carried out on grey-red soil in the valley of the river Raša (Istria). Biostimulators were applied on indeterminate, high beef tomato variety 'Signora F1' with a training system on one or two branches. PE mulch foil, fertigation, and trellis were used on the plantation. The influence of the triple treatment on fruit number, individual fruit weight, yield distribution, time of disease onset, and branch length was examined. The results of the study show that growing with two side shoots and treating with Megafol is the superior combination in terms of yield. On the other hand, the cultivation form with one side shoot in combination with Megafol leads to a better distribution of yields.

Keywords: *Solanum lycopersicon L., Humates, Lactates, Bacillus subtilis, Megafol.*

Introduction

Plant biostimulators contain plant substances and/or microorganisms, whose function when applied to a plant or rhizosphere is to stimulate the natural process of absorption and effectiveness of nutrients, tolerance to abiotic stresses and crop quality. They are often used for foliar application, but also for the rhizosphere in addition to the standard fertilization treatment. In this way, they stimulate and strengthen plant metabolism through the roots (Mešić et al., 2022). Du Jardin (2015) mentioned different categories of biostimulators classified by composition: (1) humic and fulvic acids, (2) amino acids, protein hydrolysates and other nitrogen compounds, (3) extracts of seaweed and algae, (4) inorganic biostimulators, (5) beneficial (mycorrhizal) fungi, (6) beneficial bacteria.

The application of bioregulators was studied with the aim to improve both the nutrient balance and plant growth on tomato cultivation in a greenhouse and on the field. According to previous investigations, humates, lactates and Bacillus subtilis as well as algae, seem to be particularly suitable in this respect. The special function of humus and the humic acid in the rhizosphere of plants is known, but the roots have to develop in hydroponic (soilless) systems with a few amount in ‘substrate culture’ or without the influence of humic acid, in ‘water culture’. Humic acids can accelerate the plant-growth, stimulates the process of the formation of plant organs, increases the unspecific resistance of the plants against stress conditions like high temperature, frost, drought, strong radiation (Faust, 1999). Humates have an influence on the nutrient uptake and the respiration process, the amount of sugar and amino acids, further reduce the accumulation of nitrate and makes the plants resistant to diseases and viruses (Levinsky, 1996; Böhme et al., 2005). It was found that, under certain conditions, humic acid applied to the root zone had beneficial effects on plant development (Tattini, 1990; Böhme and Hoang, 1997). According to previous investigations (Böhme et al., 2008), lactates (salts of lactic acid) seem to have also a bioregulatory effect. The application of lactates was tested as an approach to improve both nutrient balance and plant vitality. Investigations have shown that lactates have more stable bonds with several metal ions than other chelates do. Therefore lactates have been used as fertilisers and as bioregulators. Lactates are available from a Bulgarian company Ecofol (LACTOFOL®). That suspension fertiliser was designed mainly for foliar application (Pavlova and Batschvarov, 1992; Shaban et al., 1995). Hardly any information has been available so far about the effects of lactate applied to the root zone (Böhme et al., 2008). Many microorganisms from the rhizosphere can influence plant growth and plant health positively and are therefore often referred to as “plant growth-promoting rhizobacteria” (Schippers, 1992). In previous investigations, we found beneficial effects in soilless culture systems of the gram-negative rhizobacterium FZB 24® regarding the reduction of salt stress (Böhme, et al. 2005). These positive effects under salinity conditions could be confirmed by Bochow et al. (2002) in open-field research with eggplant and bell pepper in Egypt. The cultivation of different vegetables in hydroponic systems in greenhouses is quite problematic as to the proper balancing of EC and pH values. The three groups of biostimulators were therefore investigated in greenhouse experiments with the aim to stabilize in particular the chemical properties of the rhizosphere. On the field are also many stress factors for tomato plant growth caused by climate and environmental conditions, pests and diseases and unfavourable conditions in the rhizosphere with an application of biostimulators it is possible to reduce such stresses for plant growth. The field experiment was the intended to show the effects oftwo biostimulators. The first is Čudomiks an organic liquid biostimulator and plant enhancer from the "Tilurium Organic" line, a Croatian product developed by OPG Pezelj from Trilj, which is also accepted for ‘organic’ production following the EU rules. Čudomiks consists of biologically active compounds and elements extracted from plants that protect crops from fungal and bacterial diseases. The second biostimulator used is Megafol Valagro®, from Italy it promotes vegetative growth during environmental stress, stimulates plant growth and improves the effectiveness of treatments. This biostimulator contains 28% of amino acids of exclusively plant origin contains a line of vitamins, amino acids and proteins, betaines and growth factors. When applied in times of stress (frost, root asphyxia, weeding, hail), its synergistic action of betaine and amino acids allows plants to quickly and spectacularly overcome stress and improve growth (Megafol, 2022)

Material and Methods

Plant material

In both experiments indeterminate tomato (*Solanum lycopersicon* L.) was investigated. In the greenhouse experiment of the Humboldt University of Berlin, Research station Berlin-Dahlem cv. Ferrari RZ was used and in the field experiment near to Trget in Istrien, Croatia the Italian cv. Signora F1 ESASEM, a hybrid beef tomato for loose harvesting, resistant to TSWV. This cv. can be cultivated in a greenhouse and on the field.

Investigations on protected cultivation in Germany

The experiments were performed in the period from May through end of August 2011. Average temperature during the day was in average 27.5°C and during the night 23.5°C. The air humidity was between 50 and 76%. Ten tomato plants per treatment were used, cultivated in Mitcherlich pots until they had 11/12 leaves. Experimental conditions:

- The Mitcherlich pots (6L) were filled with Perlite, with an average dry density of 120 kg * m^{-3} and a grain size between 0.06mm and 1.5mm.
- K-humate with 0.01% (Fa. Humisolv) was used as the humic acid preparation.
- The Lactate (LACTOFOL ®) used in the experiments concentration: 0.08%) was added to the solution, and the lacking nutrients were supplemented
- The strain Bacillus subtilis FZB 24 of Fa. Arbitep applied as spore suspension (concentration of 10^5cfu/ ml.

The tomato plants were assessed once a week to follow up their growth and development, shoot fresh and dry matter and root fresh and dry matter as well the root length, were recorded in the final assessment. A micro irrigation was used, whereby the nutrient solution was calculated with the "Hydrofer" fertilization program in all variants (Böhme, 1993).

The stress variants consists of three levels of salt concentration EC 1.0, 3.0, and 8.0 mS cm^{-1} and pH value of 5.0, 5.8, and 7.5.

Investigations in field cultivation in Croatia

The soil of the experimental plot belongs to the characteristic type of alluvial soils in the river valleys, it is composed of clay and a lot of sand particles with a pH of 6.5-7.2 whereas the precipitation ranges from 800 to 1100 mm. Temperatures in the summer months are suitable for growing tomatoes, the difference between day and night temperatures during the summer are on average 18 ° C, and up to 30 ° C daily.

Plant density was 4.8 plants per square meter in double row, the planting distance was 90 cm between the double row beds The bed was covered with black mulch foil, the row spacing was 30 cm between rows and within the row. The effect of the two biostimulators Chudomiks and Megafol was investigated.

A different number of biostimulator treatments; 1x, 2x and 3x on tomatoes grown on one and two branches, in 14 different variants in three repetitions, a total of 42 individual plots. All variants are represented in three repetitions with a random arrangement of plots. The size of the experimental field is 5300 m^2, the size of an individual plot 126 m^2. Megafol treatment was carried out according to the instructions in a concentration of 0.33% (1 L of Megafol per 300L of water). Chudomiks was applied in the recommended dilution of 1.0%, according to the instructions on the declaration, 0.5 L of biostimulator per 50L of water. The parameters monitored in the experiment are the plant height, the number of fruits per plant, the weight of individual fruits and yield per plant.

Data evaluation

All data of the experiments were evaluated with the statistical software SPSS. Mean values and standard deviations were calculated and analysed using ANOVA - in greenhouse: LSD test, significance level P≤0.05 and in field experiment Tukey test, significance level P≤0.05.

Results and discussion

Experiments in greenhouse

The treatments showed visible effects, the results for shoot and leaf dry matter (SDM) are in line with what had been expected - highest values at EC = 3 mS ∗ cm^{-1}, lower at 1 mS ∗ cm^{-1}, and smallest at 8 mS ∗ cm^{-1} (Table 1). Compared with the control, treatments at 3 mS ∗ cm^{-1} produced only a small effect. Significantly better results were only obtained in the variant with humic acid (HA) + *Bacillus subtilis* (BS). BS and its combination with HA gave significantly better SDM also in case of nutrient deficiency, i.e. at an EC of 1 mS ∗ cm^{-1}.

The results obtained at the extremely high EC of 8 mS ∗ cm^{-1} were in line with expectations. Addition of HA, BS and HA + BS caused a significant enhancement of tomato plant development. The effect of HA and BS on root development (Table 1) is similar to that on SDM. Significantly higher root dry matter (RDM) was recorded above all with EC values of 3 and 8 mS ∗ cm^{-1}, respectively. Results are somewhat different for root length (RL). In the nutrient deficiency variant (EC = 1 mS ∗ cm^{-1}) all plants had longer roots than the control, but only slightly different root lengths were recorded if EC was higher.

Table 1. Parameters of tomato plants treated with Humic acid, Lactate and/or Bacillus subtilis at EC 1, 3 and 8 mS cm^{-1} respectively

Variants	Shoot dry Matter [g/plant]			Root dry Matter [g/plant]			Root length [m]		
	EC 1	EC 3	EC 8	EC 1	EC3	EC8	EC 1	EC 3	EC8
Control	16.67b	25.27b	5.97c	3.02a	2.94ab	0.86b	56.89b	136.81a	32.89b
Humic Acid (HA)	16.92b	23.43b	16.15a	2.72b	3.45a	1.74a	115.25ab	109.47b	47.43a
Lactate (LA)	13.63c	22.01c	5.54c	2.37b	2.91ab	0.62b	114.75ab	68.52c	35.82b
Bacillus subtilis (BS)	18.83a	23.98b	14.43b	3.27a	3.48a	1.61a	135.44a	138.21a	41.63ab
HA+BS	18.05a	27.08a	16.07a	2.9b	3.63a	1.84a	131.69a	127.78ab	52.96a
LA+BS	13.29c	15.69d	5.39c	1.93c	2.2b	0.66b	86.94b	47.04c	31.75b
HA+LA+BS	13.26c	17.11bd	5.39c	1.96c	2.74b	0.62b	90.17b	66.76c	35.65b

Different letters indicate significant differences (LSD, P=0.05).

If pH was low (pH 5.0), all treatments showed higher SDM than the control, a fact that was particularly obvious in the combination HA + LA + BS (Table 2). Particularly noticeable are the significantly better result at pH 5.8 (which level is considered optimal) and with BS.

Table 2. Parameters of tomato plants treated with Humic Acid, Lactate and/or Bacillus subtilis at pH 5, 5.8 and 7.5, respectively

Variants	Shoot dry Matter [g/plant]			Root dry Matter [g/plant]			Root length [m]		
	pH5	pH 5.8	pH7.5	PH 5	pH 5.8	pH 7.5	pH 4.5	pH 5.8	pH7.5
Control	11.0b	8.1c	2.0c	1.6a	0.7c	0.1c	31.3a	48.00b	5.7c
Humic Acid	11.2b	9.5c	4.2b	1.6a	1.0b	0.4b	41.3a	50.88ab	14.2b
LACTOFOL	9.6c	10.2b	8.7a	1.5a	1.4a	1.9a	41.9a	68.70a	24.8a
Bacillus subtilis	12.8a	9.5c	2.2c	1.6a	0.8	0.09c	32.3a	55.90ab	4.6c
HA+BS	12.7a	11.1b	2.9c	1.5a	1.2a	0.5b	37.9a	49.73b	10.2b
LA+BS	9.4c	10.8b	9.4a	1.3b	1.3a	1.9a	36.9a	47.15b	26.7a
HA+LA+BS	10.3b	15.1a	10.1a	1.7a	1.5a	1.4a	34.5a	64.35a	25.9a

Different letters indicate significant differences (LSD, P=0.05).

The results at pH 7.5, a value that is extremely high for tomato, are unambiguous.
It can be conclude, humic acid encourages the longitudial growth of plant roots, a fact that has been established at the various ECs used in the experiments. These results are in line with the findings of Tattini et al. (1990) who found that humic acid led to greater length of the root system and a larger number of lateral and hair roots. This might also explain why at an EC of 1 mS * cm^{-1} root dry matter did not increase in the same way as the root length. The beneficial effect of HA at 8 mS * cm^{-1} may also be due to its high sorptive capacity. All variants with LACTOFOL® developed significantly better than the control and also better than the other treatments. Root dry matter revealed even more obvious effects of the LA variants. This experiments, too, makes it clear that it would be advisable to record not only RDM but also root length. Here again, root length was higher in the LA variants and - like for RDM - not only at pH 7.5 but also at the optimal value of pH 5.8. The positive effect of BS on the development of tomato plants with excessive or deficient nutrient supply, i.e., the variants with EC 1 and 8 mS * cm^{-1}, respectively, may be explained by the formation of enzymes that interfere with the nutrient balance or produce a general vitalizing effect. This applies also to the beneficial effects of BS at different pH values. Similar assumptions were made by Bochow et al. (1995) in his interpretation of experiments with different vegetable species. Combinations of BS and HA turned out equally effective at suboptimal EC and pH values. The interactions involved will have to be investigated in future experiments.

Experiments in the field

In the experiment, a statistically significant difference in plant height was found depending on the cultivation form. A tomato grown on one branch formed a significantly longer branch compared to a tomato grown on two stems (Figure 1). Biostimulators and the number of treatments with biostimulators did not significantly affect the height of tomatoes.

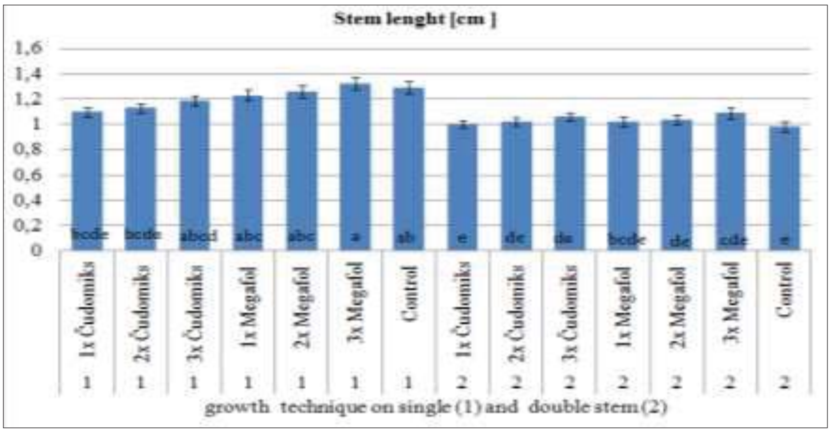

Figure 1. Influence of cultivation form, number of treatments and type of biostimulator on tomato stem height (Different letters denote significance, Tukey test, p <0.05)

The number of fruits per plant is influenced by the cultivation practice, the type of biostimulator and the number of treatments. Statistically confirmed, the largest number of fruits per plant was found in variants with double and triple treatment of Megafol in the cultivation form of tomatoes on two branches. A significant increase in the number of fruits was also statistically confirmed in comparison with the treatments with Čudomiks list (Figure 2).

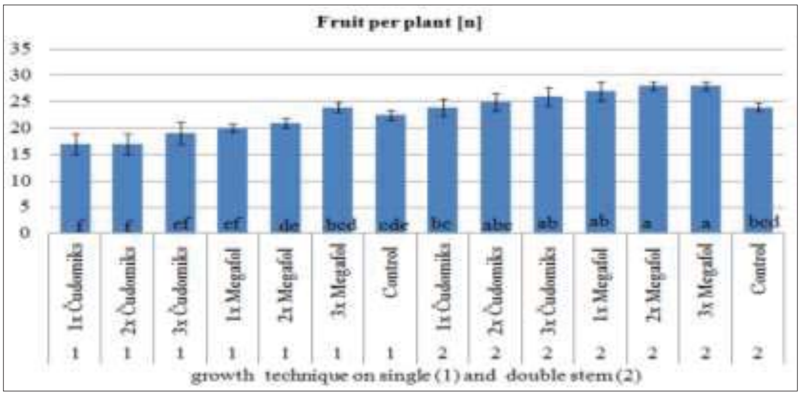

Figure 2. Influence of cultivation form, number of treatments and type of biostimulant on the number of fruits per tomato plant (Different letters denote significance, Tukey test, p <0.05)

In the cultivation form on two branches, it was found that triple treatment with biostimulators significantly affected the weight of an individual fruit compared to a smaller number of treatments. In the total yield per plant, statistically confirmed the best variant was triple treatment with Megafol in tomato cultivation on two branches, in second place in the amount of yield per plant was achieved in the variant triple treatment with Megafol in tomato cultivation on one branch (Figure 3).

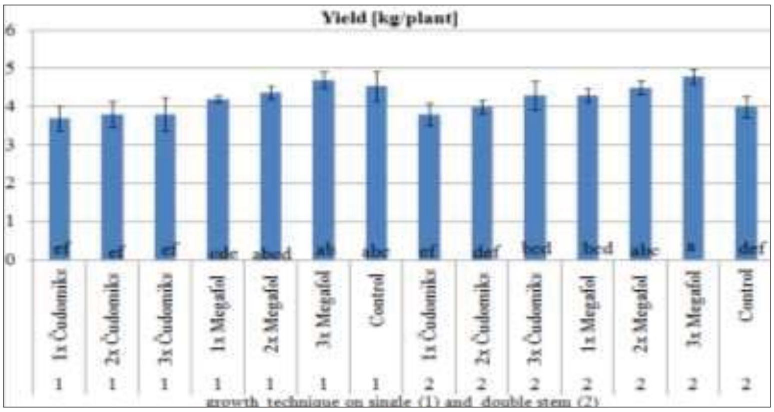

Figure 3. Influence of cultivation form, number of treatments and type of biostimulators on yield per plant (Different letters denote significance, Tukey test, p <0.05)

The results of research on the influence of biostimulators in tomato cultivation are in line with research on the application of biostimulators in nettle Radman et al. (2022) who found that biostimulators based on amino acids and humic acids did not have a justified effect on morphological indicators (plant height, weight, number of nodules and leaves), which was shown in tomato cultivation, where a significant influence on growth height had a cultivation form, neither the number of treatments nor the type of biostimulator. Research by Klokić et al. (2020) using biostimulators, Megafol and Viva, with different dominant compositions (amino acids and humic acids) confirm positively affected yield in two cultivars of semi-determinate tomato (cv. Gravity F1 and cv. Minaret F1) which is consistent with this research. Namely, proline is an amino acid, which is added to plants by the application of Megafol®. This amino acid is characterized by the ability to remove free radicals (Khedr et al. 2003), better antioxidant ability to respond to stressful growing conditions resulting in better plant condition (Kaul et al., 2008) and consequently reflected in higher plant productivity, in the formation of higher number of fruits. Parađiković et al. (2010) confirm the effects of the use of Megafol in pepper cultivation on fruit quality and the occurrence of peak rot. The effectiveness of Megafol biostimulators is also contributed by the content of tryptophan, which is a precursor in the synthesis of melatonin, which according to research by Arnao and Hernandez-Ruiz (2006) strengthens the ability to remove free radicals in metabolic processes and is helpful to improve the productivity of plant growth. Melatonin biosynthesis in plants is influenced by tryptophan and IAA (indole-3-acetic acid.

Conclusion

Tomato as one of the most famous types of vegetables in the world in cultivation is very demanding and it takes a large number of working hours to obtain the desired quality and quantity. In the experiments, the cultivation of indeterminate tomato cultivars in greenhouse and in the open field makes visible the possibility of strengthening plant growth and yield, by use of different biostimulators in particular in in stress situations.

Following the results in the greenhouse experiments can be conclude, addition of organic and/or biological agents to the substrate or nutrient solution helps to minimize stress situations and, hence, adverse effects on plant growth that are due to suboptimal EC or pH values. The humates, lactates and Bacillus subtilis have different bioregulatory effects. The combination of Humates

and BS is already produced and offered from the company Humintech under the name BioHealth® BS WSG product because the very effective bioregulatory effects.
Lactate (LACTOFOL®) was found to be also suitable as a fertilizer for making up nutrient solutions to be used in hydroponic systems. However, its stress-reducing effect is more pronounced at non-optimal pH values than at suboptimal EC.
In the field experiment was visible that the deviations in fruit quality between treatments were very small, but in quantity deviations were somewhat larger. In the variants of the cultivation form on one branch, the treatment with 3 times the biostimulator Megafol proved to be the best. In the two-branch cultivation form, the best variant was also a three-course treatment with Megafol. However, according to the fruiting schedule, cultivation on one branch was more favourable. Growing on two branches increases the number of fruits and total yields, but the potential for the occurrence of fungal diseases is increased due to the higher density of plantations. From the economic point of view, the use of Megafol biostimulants is economically viable due to the increase in yield and fruit quality.

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