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## The influence of biostimulants on strawberries yield, nutritional and sensory fruit quality

### Abstract

The study was conducted as open field trial, with irrigation, with the Clery strawberry variety. The aim of the study was to investigate the effect of foliar application on berry and seaweed extract on the yield and quality of strawberry fruit (sugar, acid and anthocyanin content). The experiment was set up by random block system in 4 repetitions. The following treatments were applied to the strawberry crop: 1. control, 2. conventional NPK fertilization (10-52-10), 3. 30% reduced conventional NPK fertilization, 4. 30% reduced conventional fertilization + biostimulants. Due to arid growing conditions, NPK fertilizer was applied foliarly. Statistical analysis of the data showed significant differences between treatments in strawberry yield, and anthocyanins content. Compared to the control, the biostimulator had an effect on strawberry yield and total anthocyanin content, but not on the total soluble sugar and acid content of strawberry fruit.

**Keywords:** strawberry, biostimulant, fruit quality

### Introduction

Strawberry (Lat. *Fragaria* × *ananassa* Duch.) is the most significant berry fruit with fruit qualities that can be used for various purposes. Also, strawberries ripen in early spring, when the market is poorly stocked with fresh fruits, so the demand for strawberries has been increasing lately. Production of this fruits in the Republic of Croatia is not developed to its full potential and the demand is higher than the supply, so some of the strawberries are imported into the Croatian market (Voća et al., 2008). Due to its nutritional compounds (antioxidants, phenols, anthocyanin) and sensory characteristics (flavor, color, sugar and acid content), it has become a topic of scientific research that goes towards increasing these properties. The sugars and acids of strawberries are regarded as significant quality factors by consumers and the food industry (Kallio et al., 2000). Sturm et al. (2003) claim that parameters like total acids (TA), total soluble solids (TSS) and their ratio are very important in determining strawberry fruit quality. Lopes da Silva et al. (2007) investigated the nutritional properties of strawberries and concluded that antioxidant capacity in berries seems to be mostly due to the activity of phenolic compounds, such as anthocyanins, and antioxidative vitamins, mainly vitamin C. In modern strawberry production, there are numerous strategies for enhancing the nutritional and sensory properties of strawberries, one of which is the use of biostimulants. According to Parađiković et al. (2019), biostimulants can be applied at all plant growth stages, from germination to full plant and fruit commercial maturity using the seed treatment, foliar application or irrigation.

Biostimulants are defined in EU regulation (2019) as products that can be used in agricultural production to improve nutrient consumption efficiency, tolerance to various abiotic stresses, and increase the availability of restricted nutrients in the soil or in the rhizosphere. Plant biostimulants are various substances and microorganisms used to promote plant growth

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and can be classified into 5 groups: microbial inoculants, humic acids, fulvic acids, protein and amino acid hydrolysates, and seaweed extracts (Calvo et al., 2014). Their application at low concentrations enhances nutritional efficiency, abiotic stress tolerance, and / or crop quality traits, regardless of its nutrients content (de Vasconcelos et al., 2019). The aim of the study was to determine the influence of biostimulants on growth and development, yield components and quality properties of strawberries grown in the open field.

## Materials and methods

The trial was set up as a preliminary study in an open field with irrigation in Klisa (east part of Croatia) at the trial field of the Faculty of Agrobiotechnical Sciences Osijek. It was set up in four repetitions as randomized block design and following parameters were monitored: yield components, fruit sensory and nutritional quality (total soluble sugar, acid and anthocyanin content).

Weather conditions in the spring of 2019 in the Republic of Croatia were characterized by the changeable weather with excess rainfall, especially in May, followed by a dry period, so the planting of container strawberries (Clery cultivar) was carried out on 19. 06. 2019. The foil was unfolded and 24 trial plots were placed with a 40 cm spacing between the plots (210 cm length). The width of the plot was 80 cm. 7 strawberry seedlings were planted on each plot (7 plants/plot). All plots were treated with following treatments: 1. UTC - control, 2. Conventional PPP, 3. Conventional PPP - 30%, 4. Conventional PPP - 30% + biostimulants, strategy 1.

Conventional PPP understood the application of foliar NPK fertilizer formulation 10-52-10 in the amount of 30 g per 5 l of water, the total amount of applied fertilizer were 60 g. Conventional PPP- 30% understood 30% reduced fertilization or the application of foliar NPK fertilizer formulation 10-52-10 in the amount of 21 g per 5 l of water, total applied amount were 42 g. The application of NPK foliar fertilizers was necessary due to bad weather conditions (drought stress) and late planting and strawberry seedlings had to be supplemented with nutrients. Foliar fertilizer was not applied together with biostimulants but separately. Biostimulant understood application based on extract of berries and seaweed. Biostimulant was added in an amount of 170 ml per 1 liter of water and foliar applied to Conventional PPP treatments - 30% + biostimulants, strategy 1. Dates of biostimulant application were 03.07.2019, 11.07.2019 and 23.07.2019. Strawberry picking was done on July 26, 2019. Biostimulant is still in the research phase *in vitro* and therefore does not have a full name.

### *Laboratory analyses*

All plant (fruit) analyses were done in Laboratory for plant analyses at Faculty of agrobiotechnical sciences Osijek Croatia during the 2019.

The strawberries fruits were collected from all trial plots for determination of yield. Total soluble sugars (TSS) and acids (TA) content as well as anthocyanin content were determined in the strawberries fruits. The TSS in this research was determined by refractometer while the TA was determined by potentiometric titrations. The refractometer, which optically measures the refractive index of juice, is the standard method used to measure TSS of fruit and vegetables and the results are expressed as Brix (Alamo et al., 1993). The usual method for the determination of TA in fruit juices is based on the titration of the sample with 0.1 mol/l NaOH, using phenolphthalein as indicator, or potentiometric detection in the case of heavily colored samples, and results are expressed as g of organic acid equivalent per 100 g fresh weight (Alamo et al., 1993). Anthocyanin content of analyzed fruits was determined by pH differential method using

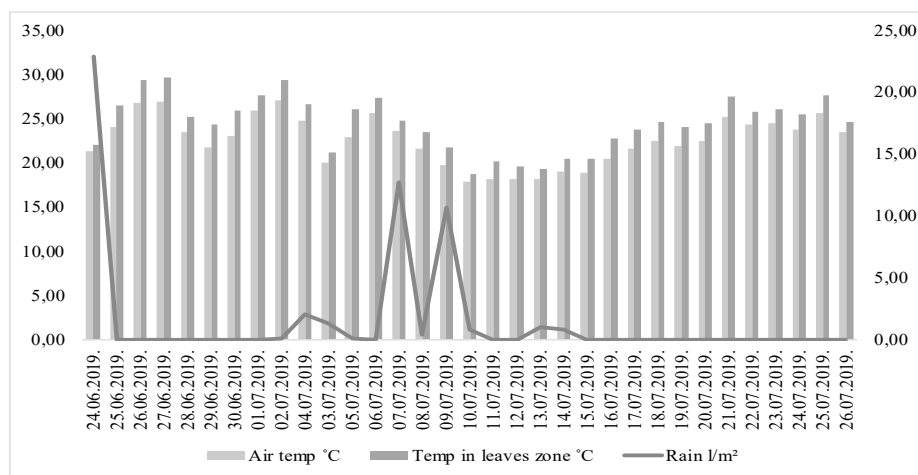
two buffer systems: potassium chloride buffer, pH 1,0 (0,025 M) and sodium acetate buffer, pH 4,5 (0,4 M) (Lako et al., 2007). Anthocyanin content of samples mg cyanidin-3-glucoside/100 g of fruit was calculated by following equation:  $ACC = (A \times M \times DF \times 100 / \epsilon)$  (1).

All determinations were made in triplicate and the results were statistically processed in Microsoft Excel and SAS 9.3 software package (SAS Institute Inc., NC, USA).

## Results and discussion

### Weather conditions

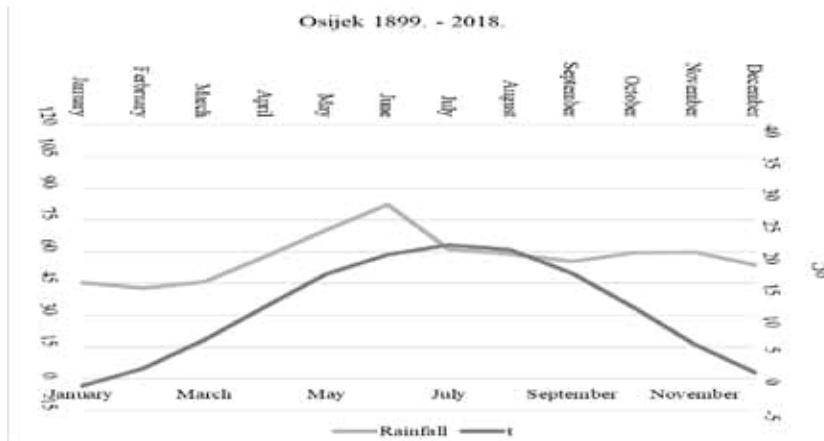
Rainfall and temperature were measured at Tenja-Klisa Weather Station and according to that measurement the average air temperature in June was 23,8°C and in July 22,1°C with more rainfall at the beginning of June (Figure 1).



**Figure 1.** Rainfall and temperature during strawberry vegetation at field trial  
**Grafikon 1.** Oborine i temperature tijekom vegetacijskog ciklusa na pokusu jagoda  
Source/Izvor: Croatian Meteorological and Hydrological Service, 2019.

The average temperature in spring 2019 was within the perennial average of the Republic of Croatia and analysis of the precipitation amounts for June 2019 expressed as percentages (%) of 1899 - 2018 average shows that these precipitation amounts were mainly below the average. A comparison with the multi-annual average reveals that precipitation amounts for June 2019 were under 50 % (Figure 2).

According to Perin et al. (2019) the frequency of the periods of drought and limited water availability has negatively affected the agricultural production. While rainfall was low during this period in June, there was still enough of water in the soil, so the strawberry planting and growing was successful.



**Figure 2.** Precipitation and mean air temperature over multiyear periods for Osijek  
**Grafikon 2.** Višegodišnji prosjek oborina i prosječne temperature zraka za područje Osijeka  
 Source/Izvor: Croatian Meteorological and Hydrological Service, 2019.

#### Plant analyses

The use of biostimulants has affected the strawberries yield and the highest yield (g/plant) was determined for Conventional PPP – 30% + biostimulants, strategy 1 and it was significantly different according to UTC – control treatment. The statistically lowest yield was determined on the UTC - control and Conventional PPP (Table 1). The obtained results agree with the study of Mattner et al. (2018) who obtained an increase in root density and strawberry yield by applying commercial Seasol® extract (extracted from the seaweed *Duvillaea potatorum* and *Asco-phylum nodosum*) to two strawberry cultivars (Albion and Fortuna). By growing strawberries on a chlorosis-inducing substrate due to Fe deficiency, Spinelli et al. (2010) examined the effect of foliar application of seaweed extract at two different concentrations in one two and three applications. The used extract increased the mass of the aboveground part of the plant, root and fruit. Alam et al. (2013) treated a crop with varying amounts of seaweed extract *Asco-phylum nodosum* (soluble powder of *Asco-phylum* extract (SAEP)). Application of 1, 2 or 4 g of L<sup>-1</sup> extract over 8 weeks yielded the highest fruit yields at treatments 1 and 2 g of SAEP L<sup>-1</sup>.

**Table 1.** Statistical analysis of strawberries yield and quality parametars

**Tablica 1.** Statistička analiza prinosa i kvalitativnih svojstava jagode

Treatments <i>Tretmani</i>	Yield (g/plant) <i>Prinos (g/biljci)</i>	Total soluble solids (TSS) ° Brix <i>Ukupni topljivi šećeri ° Brix</i>	Total acid (TA) (%) <i>Ukupne kiseline (%)</i>	Total anthocyanins (mg/100g) <i>Ukupni antocijani (mg/100g)</i>
1. UTC - control	17.32 B	12.30 A	0.82 A	22.90 B
2. Conventional PPP	17.95 AB	13.23 A	0.81 A	23.08 B
3. Conventional PPP - 30%	18.19 AB	13.96 A	0.70 A	23.02 B
4. Conventional PPP - 30% + biostimulants, strategy 1	19.52 A	14.71 A	0.75 A	25.50 A
Minimum	17.32	12.30	0.70	22.90
Maximum	19.52	14.71	0.82	25.50
Standard Deviation	1.3164	1.8356	0.2118	1.1513
LSD	1.8592	2.8519	0.3667	0.6877

A,B Means with the same letter are not significantly different,  $p \leq 0.05$

The sugars and acids, together with small amounts of dissolved vitamins, fructans, proteins, pigments, phenolics, and minerals, are commonly referred to as soluble solids. Total soluble solids (TSS) are the most important quality parameter used to indicate sweetness of fresh and processed horticultural food products, in laboratories for research and by industry to determine marketing standards (Magwaza and Opara, 2015). The acidity of a juice (TA) is due to the content of several organic acids (i.e. citric, malic, fumaric, acetic, ascorbic, galacturonic). The acidity of the fruit juice is relevant to keep the organoleptic nature inalterable and to avoid fermentation processes (Xu et al., 2008).

Regarding total soluble solids, the highest TSS content was determined on Conventional PPP treatment, strategy 1, with no significant difference found among the treatments. Also, there was no statistically significant difference between treatments for total acid content. Similar results were obtained by Roussos et al. (2009) who treated a strawberry crop with a mixture of seaweed extract and a commercial mixture of nitrophenolate and with a mixture of auxin (phenothiol) and gibberellic acid in two doses. Biostimulants increased the yield and size of the fruit, but had no significant effect on fruit juice pH, acidity, and overall solubility concentration. Furthermore, they had no significant effect on the concentration of organic acids and carbohydrates in the fruit and on the color of the fruit, although they increased the total anthocyanin concentration. For total anthocyanin, a statistically significant difference was found between Conventional PPP alternate to biostimulant, strategy 1 (highest anthocyanin level) and all other treatments while the lowest anthocyanin content was determined on three treatments: UTC-control, Conventional PPP - 30%, Conventional PPP (Table 1). Foliar application of GUMI 20K fertilizer and biostimulant Mival-Agro, Stimolante 66f, Alga mix B Mg in three strawberry varieties Prichko et al. (2014) obtained higher fruit mass, dry matter and sugar content (7-10%), organic acids (10-15%), vitamin C (9-14%) and P-active substances (3-12%). According to Frioni et al. (2018) the seaweed extract did not affect grapes leaf gas exchanges, yield or cluster and berry size, but hastened veraison, improved anthocyanins accumulation in all cultivars and increased phenolic content particularly in Sangiovese. The same authors claim that medium-late application of the seaweed extract can be a simple way to favour chromatic and chemical proprieties of grapes and wines.

## Conclusions

The study showed that using biostimulants can be a promising strategy to improve strawberries yield and nutritional quality by increasing of antioxidant compounds. According to the results obtained, biostimulant had an effect on strawberry yield and the content of total anthocyanins compared to the control. However, the use of biostimulants did not affect the total soluble sugars and acids content in strawberries fruits. In combination with appropriate fertilization and irrigation, biostimulants can be a good solution to achieve stable strawberry production with high nutritional values. This is very important for further research towards finding a balance between biostimulant/fertilizer doses and improving the quality of strawberry fruit, which will contribute to the ecological and economic balance of production.

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## Utjecaj biostimulatora na prinos, hranjivu i senzornu kvalitetu plodova jagoda

### Sažetak

Istraživanje je provedeno u poljskim uvjetima, uz navodnjavanje, sa sortom jagode Clery. Cilj istraživanja bio je ispitati utjecaj folijarne primjene biostimulatora na bazi ekstrakta bobica i morskih algi na prinos i kvalitetu ploda jagode (sadržaj šećera, kiselina i antocijana). Pokus je postavljen po slučajnom blok sustavu u 4 ponavljanja. Na usjevu jagoda primijenjeni su tretmani: 1. kontrola, 2. konvencionalna gnojidba NPK (10-52-10), 3. 30% reducirana konvencionalna gnojidba NPK, 4. 30% reducirana konvencionalna gnojidba + biostimulator. Zbog sušnih uvjeta uzgoja NPK gnojivo primijenjeno je folijarno.

Statističkom analizom podataka dobivene su značajne razlike između tretmana u prinosu ploda jagode i sadržaju ukupnih antocijanina. U usporedbi s kontrolom biostimulator imao je utjecaj na prinos jagode i sadržaj ukupnih antocijanina, ali ne na sadržaj šećera i kiselina u plodu jagode.

**Ključne riječi:** jagode, biostimulator, kvaliteta ploda