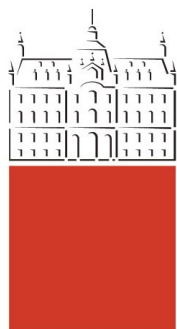


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KRŠKO, 17. – 18. JANUAR 2024

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## **DIHYDROQUERCETIN-BASED NANOEMULSION AS A PROMISING BIOSTIMULANT FOR ENHANCING IN FRUIT QUALITY OF STRAWBERRY**

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### **ABSTRACT**

Natural biostimulant products that enhance the growth, productivity, and nutritional quality of fruits are becoming increasingly popular, particularly in challenging environmental conditions like fluctuating temperatures, spring frosts, and excessive precipitation. This study aimed to evaluate the effect of the dihydroquercetin-based biostimulant on the yield and fruit quality of the strawberry cultivar 'Alba' grown in an open field condition. Biostimulant application increased strawberry yield, total phenolic content and antioxidant activity, while physical traits, total soluble solids (TSS), titratable acidity (TA), pH, and total sugars (TS), invert sugar (IS) and sucrose (SUC) responded differently to the biostimulant. The application of dihydroquercetin-based nanoemulsion negatively affected the content of TSS, TA, TS and IS but not the external appearance of the strawberry (weight, dimensions, shape, index color and firmness). These data suggested that dihydroquercetin-based treatment might maintain physical traits while improving the content of bioactive compounds of strawberry fruit due to the enhancement of the phenolic content and antioxidant activity.

**Key words:** biostimulant, strawberry, yield, physical traits, fruit quality

### **NANOEMULZIJA NA OSNOVI DIHIDROKVERCETINA KOT OBETAVNI BIOSTIMULANT ZA IZBOLJŠANJE KAKOVOSTI PLODOV JAGODE**

### **POVZETEK**

Naravni biostimulanti, ki izboljšujejo rast, rodnost in notranjo kakovost plodov, postajajo vse bolj pomembni, zlasti pri spreminajočih se klimatskih spremembah, kot so nihanje temperatur, spomladanske pozebe in prevelike količine padavin. Namen te študije je bil oceniti učinek biostimulanta na osnovi dihidrokvercetina na rodnost in kakovost plodov žlahtnega jagodnjaka sorte 'Alba', gojenega na prostem. Uporaba biostimulanta je povečala pridelek, vsebnost skupnih fenolov in antioksidativni potencial, medtem ko so se druge spremenljivke, kot so topna suha snov (TSS), titracijske kisline (TA), pH, vsebnost skupnih sladkorjev (TS), invertnega sladkorja (IS) in saharoza (SUC), različno odzvale na uporabo biostimulanta. Uporaba nanoemulzije na osnovi dihidrokvercetina je negativno vplivala na vsebnost TSS, TA, TS in IS, ne pa na zunanji videz ploda (maso, velikost, obliko, barvo in trdoto). Ti podatki kažejo, da bi lahko uporaba tega biostimulanta ohranila fizikalne lastnosti,

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hkrati pa povečala vsebnost bioaktivnih snovi v plodovih s povečanjem vsebnosti fenolov in antioksidativne aktivnosti.

**Ključne besede:** biostimulant, jagoda, pridelek, fizikalne lastnosti, kakovost ploda

## 1. INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.) is a fruit with a desirable taste and unique flavor. Attractive characteristics of strawberry fruits include aroma, taste, color, texture and bioactive compounds including minerals, vitamins, antioxidants, and secondary metabolites (Aharoni et al., 2002). Given that strawberries can be stored for a short time and are mainly consumed fresh, interest in identifying alternate safe preparations to preserve the nutritional and medicinal properties of fresh fruits. In recent years, many new strategies have been developed to improve sustainable production in horticultural crops, including biostimulants, which improves fruit quality, nutrient use efficiency, and tolerance against abiotic stress (Colla et al., 2015; Rouphael and Colla, 2020). The main categories of plant biostimulants include natural compounds such as humic and fulvic acids, protein hydrolysates, seaweed extracts (Battacharyya et al., 2015; Canellas et al., 2015; Colla et al., 2017), beneficial fungi (e.g., arbuscular mycorrhizal fungi and *Trichoderma* spp.) (Rouphael et al., 2015), and plant growth promoting rhizobacteria (Ruzzi and Aroca, 2015). 'Botanicals' are defined as the naturally occurring secondary metabolites (phytochemicals) extracted from the plant, which can be used in pharmaceutical (drug), cosmetic (creams), food (food ingredients), and agriculture industries (plant protection) (Dimetry, 2014; Seiber et al. 2014). These compounds are generally safer than conventional chemical pesticides to humans and the environment, hence used as biostimulants (Dimetry, 2014; Ertani et al. 2013; Ziosi et al., 2012). 'Taxifolin' is a natural preparation based on dihydroquercetin with a significant content of propyl glycol, compatible with all other plant flavonoids. The main effect of the product is reflected in its antioxidant activity, which promotes growth and development, leads to timely technological and biological maturity, increase yield, and helps the plant to achieve its maximum genetic potential. While nanoemulsions have been widely studied for drug delivery and other applications, their use specifically for preserving the nutritional and medicinal properties of fresh fruits is less common. Because of their large surface area-to-volume ratio, nanoemulsions can provide a higher stability against gravitational separation and aggregation with their physicochemical and biological properties compared to the conventional emulsions (McClements, 2010; McClements, 2011).

This research focused on the effectiveness of dihydroquercetin-based nanoemulsion ('Taxifolin') and its potential to improve the yield and fruit quality of strawberries. Despite their beneficial effects in food, plant biostimulants are still poorly explored and implemented in agricultural practice. The investigation on the influence of the dihydroquercetin-based plant biostimulant is expected to assist producers in implementing more effective and reliable products for sustainable strawberry production as an alternative to traditional products.

## 2. MATERIALS AND METHODS

### Study area and experimental layout

The trial was carried out in the year 2023 in an experimental strawberry field in the Western Morava valley (Samaila; 43° 45' N, 20° 32' E, 250 m a.s.l.). The strawberry plantlets of *Fragaria annanasa* Duch. cv. 'Alba' were planted in August 2022 under field conditions using

the black plastic hill culture production system in single rows at intervals of 18 cm. In addition to standard cultivation practices, the plants were regularly irrigated through a drip irrigation system according to soil humidity. The fertilizers were applied through fertigation according to the phenological stage of the plant.

A randomized block design with three replicates was used. Along the single rows, each plot was 5.5 m in length, which corresponded to ~30 plants per plot. The plots were separated from each other by 0.5 m of untreated plants.

### **Treatments and sampling**

Strawberry plants were treated with water (control) and dihydroquercetin nanoemulsion- 'Taxifolin' (treatment) in 2023. Plants were sprayed every 5 days, from flowering to ripening.

Fruits samples were harvested at the commercial maturity stage from 90 plants (3 replicates of 30 plants). Each replicate consisted of 90 fruits (30 fruits from three harvest times: beginning, middle, and end of harvest season). Immediately after harvest, the physical parameters of the fruits were determined. After that, fruits from all three harvest times were combined into an average sample for chemical analysis.

### **Determination of fruit quality parameters**

Within physical properties of fruits, berry weight, dimensions (length and width), shape index, and firmness were performed by classical morphometric methods. Fruit weight is determined by measuring on precision scale (Mettler Toledo, USA), with an accuracy of  $\pm 0.01$  g. Fruit dimensions were measured by digital caliper (Carl Roth, Germany) with an accuracy of  $\pm 0.05$  mm. The value of the fruit shape index is obtained by calculation between fruit length and width. Fruit firmness is determined by using a CT3 Texture Analyser and (Brookfield, USA) and expressed in Newtons (N).

Fruit color was evaluated with the CIELAB color system (Commission Internationale de l'Eclairage, 1986). CIELAB-system color components  $L^*$  (lightness),  $a^*$  (red-green), and  $b^*$  (yellow-blue) were obtained with a Minolta CM-5 spectrophotometer (spectrophotometric method, D65, 30 mm  $10^\circ$ , reflection measurement, gloss excluded, Minolta, Osaka, Japan).

Total soluble solids (TSS), expressed as  $^\circ$ Brix, were measured with a digital refractometer (Pocket PAL-1, Atago, Japan). Titratable Acidity (TA) was determined by titration to an endpoint of pH-value 7.0 (0.1N NaOH) and multiplied by the acidity factor of citric acid (0.75) to express acidity as %. The content of total sugars (TS), invert sugars (IS) and sucrose (SUC) were determined volumetrically, using the Luff-Schoorl method (Egan et al., 1981) and expressed as %. pH value was measured with a pH-meter. The sweetness index was calculated as the total sugars and titratable acidity ratio (TS/TA).

Total phenolic content (TPC) was determined using a modified Folin-Ciocalteu method (Singleton et al., 1999; Liu et al., 2002). A 0.2 ml aliquot of the 40-fold water-diluted strawberry extract was mixed with 0.2 ml of 1:10 Folin-Ciocalteu reagent. The tube was allowed to stand at room temperature for 1 min. Then, 2 ml of 7.5%  $\text{Na}_2\text{CO}_3$  were added to the mixture. After 2 h at room temperature, absorbance was measured at 765 nm. The results were expressed as mg of gallic acid equivalents per 100 g fresh weight of the sample (mg GAE  $100\text{ g}^{-1}$  FW).

Antioxidant activity was determined using the DPPH (1,1-Diphenyl-2-picryl-hydrazyl) method reported by Brand-Williams et al. (1995) with modifications (Sánchez-Moreno et al., 1998). An aliquot of the fruit phenolic extract was added to the DPPH (Sigma–Aldrich, USA) solution in methanol and vortexed. A control sample, containing the same volume of solvent instead of the extract, was used to measure the maximum DPPH absorbance. The samples stayed in the dark for 30 min. The absorbance at 515 nm was recorded to determine the concentration of the remaining DPPH. The results were expressed as TROLOX equivalent per 100 g of fresh weight (mmol TE 100 g<sup>-1</sup> FW).

The data obtained in the research was processed applying the one-way analyses of variance (ANOVA, F test) at  $p < 0.05$ . The analyses were performed in three replications and the obtained values were expressed as mean  $\pm$  standard error. Means were compared with the Duncan test at  $P \leq 0.05$ .

### 3. RESULTS AND DISCUSSION

In order to preserve natural resources for future generations and produce better nutritive quality agricultural products, the usage of biostimulants based on plant extracts may be a good solution for enhancing the synthesis of bioactive compounds in plants. Biostimulants can be derived from plant extracts rich in phenolic compounds, such as certain medicinal plants or herbs. Dihydroquercetin (DHQ, international nonproprietary name 'Taxifolin') is a phytochemical (natural antioxidant or bioflavonoid) that occurs in plants of various families. It was first isolated from Douglas fir, *Pseudotsuga taxifolia*, and named after it. It has been reported that taxifolin is widely distributed in medicinal plants, like *Allium cepa* L., *Silybum marianum* L., *Catha edulis*, and *Larix gmelinii*, and glycosides of taxifolin in medically important plants, like *Garcinia epunctata*, *Hydnocarpus alpine*, *Smilax glabra*, and *Hypericum* (Thuan et al., 2022), but it is isolated in large amounts (up to 4.5%) only from Siberian larch (*Larix sibirica*) or Dahurian larch (*L. gmelinii*) (Orlova et al., 2022). Dihydroquercetin nanoemulsion did not affect the external appearance of the fruit in our study (Table 1).

Table 1: Effect of the dihydroquercetin-based biostimulant on physical properties and yield of strawberry fruit

Preglednica 1: Vpliv biostimulanta na osnovi dihidrokvercetina na fizikalne lastnosti in pridelek plodov žlahtnega jagodnjaka

	Weight (g)	Length (mm)	Width (mm)	Shape index	Plant Yield (g)
Treatment	22.8 $\pm$ 2.4 a	46.5 $\pm$ 2.5 a	34.5 $\pm$ 1.4 a	1.4 $\pm$ 0.0 a	956.3 $\pm$ 25.6 a
Control	19.8 $\pm$ 1.6 a	43.5 $\pm$ 1.8 a	32.5 $\pm$ 1.2 a	1.3 $\pm$ 0.0 a	837.7 $\pm$ 30.1 b
ANOVA	ns	ns	ns	ns	*

Different small letters indicate statistically significant differences at  $p \leq 0.05$  level (Duncan test); \* – statistically significant differences, ns – non-significant difference between treatments.

Indeed, slight increase were recorded compared to the control in the fruit weight, length, width and shape index parameters after treatment with dihydroquercetin nanoemulsion. However, it is important to note that the plant yield was significantly higher in the treatment than in the control. Plant yield was in the same range as reported by Weissinger et al. (2010). The authors evaluated early-ripening cultivars suitable for organic growing in Eastern Austria



sold on the fresh market and detected that 'Alba' turned out to be most convincing when summarizing important characteristics as tolerance to *Verticillium* wilt, high yield, high percentage of marketable fruits, and appearance. Besides, the external appearance of the fruit, including color and firmness, are fundamental quality parameters for consumer acceptance (Hernández-Muñoz et al., 2008). In this study, dihydroquercetin treatment did not significantly affect fruit color and firmness (Table 2). Fruit firmness is of vital importance at various points of the fruit supply chain, from determining harvest time, choosing packaging and transportation methods, regulating storage conditions, and predicting shelf life (Wang et al., 2023).

Table 2: Effect of the dihydroquercetin-based biostimulant on strawberry fruit color and firmness

Preglednica 2: Vpliv biostimulanta na osnovi dihidrokvercetina na bravo in trdoto plodov žlahtnega jagodnjaka

	Firmness (N)	L*	a*	b*
Treatment	0.34±0.03 a	34.82±0.65 a	43.12±0.64 a	28.77±0.40 a
Control	0.32±0.02 a	34.77±0.35 a	42.60±0.73 a	27.96±0.54 a
ANOVA	ns	ns	ns	ns

Different small letters indicate statistically significant differences at  $p \leq 0.05$  level (Duncan test); \* – statistically significant differences, ns – non-significant difference between treatments.

In recent studies, the influence of 'botanicals' on the control of pests and diseases in various fruit species was addressed. However, certain plant-based biostimulants, which contain phenolic compounds, can be applied to plants to enhance their overall health and stress response which can result in improved fruit quality. The application of microbial and non-microbial plant biostimulants can modify plant primary and secondary metabolism (Colla et al., 2015; Rouphael et al., 2015), leading to the synthesis and accumulation of antioxidant compounds (i.e., secondary metabolites), which are important for the human diet.

Table 3: Effect of the dihydroquercetin-based biostimulant on total soluble solids (TSS), titratable acids (TA), invert sugars (TS and TA), sucrose (SUC), and pH of strawberry fruit

Preglednica 3: Vpliv biostimulanta na osnovi dihidrokvercetina na vsebnost topne suhe snovi (TSS), titracijskih kislin (TA), skunih sladkorjev in invertnega sladkorja (TS in TA), saharozo (SUC) in pH plodov žlahtnega jagodnjaka

	TSS (°Brix)	TS (%)	IS (%)	SUC (%)	TA (%)	pH	TS/TA
Treatment	5.20±0.11 b	3.06±0.17 b	2.91±0.16 b	0.15±0.02 a	0.81±0.01 b	3.30±0.01 a	3.78±0.09 b
Control	6.50±0.00 a	4.08±0.00 a	3.84±0.03 a	0.22±0.03 a	0.91±0.02 a	3.29±0.00 a	4.48±0.01 a
ANOVA	*	*	*	ns	*	ns	*

Different small letters indicate statistically significant differences at  $p \leq 0.05$  level (Duncan test); \* – statistically significant differences, ns – non-significant difference between treatments.

The effect of the dihydroquercetin nanoemulsion on the chemical composition of strawberry fruit is shown in Table 3. There was a significant difference in TSS content between the

control and treated samples. The control sample exhibited 25% higher TSS, TS, IS, and TA compared to treated samples. Contrarily, the application of the dihydroquercetin-based biostimulant induced a significant increase in strawberry fruit quality in terms of antioxidant activity and total phenolic content (Figure 1). The results are in accordance with the report of Soppelsa et al. (2018), who determined a minor impact of biostimulant products based on *A. nodosum* seaweed extract, protein hydrolysates, and B-group vitamins on primary apple quality traits (size, flesh firmness, acidity, and total sugars), whereas on the other hand, they determined an improvement of the intensity and extension of red coloration in 'Jonathan' apples at harvest in the 2-year trials.

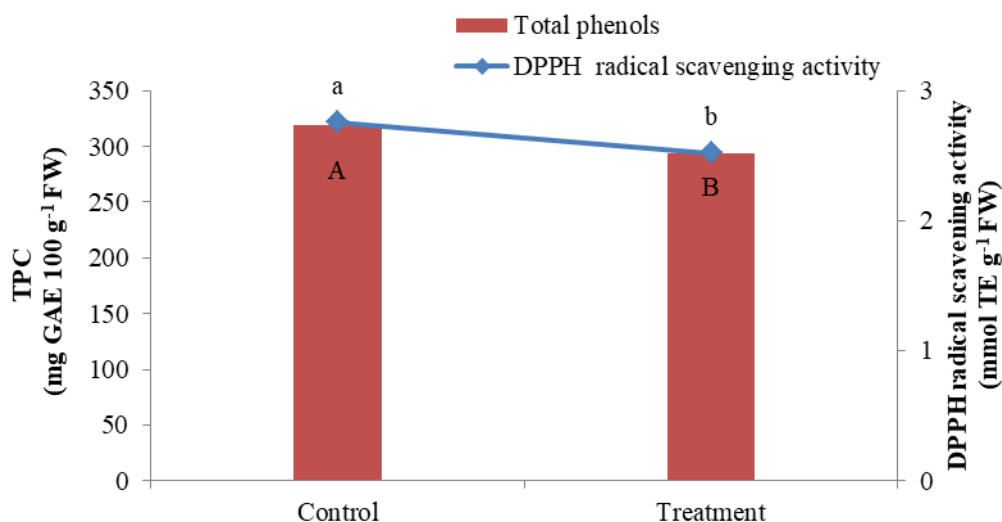


Figure 1: Effect of the dihydroquercetin-based biostimulant on total phenolic content (TPC) and antioxidant activity of strawberry fruit. Different letters on the column indicate statistically significant differences at  $p \leq 0.05$  level (Duncan test)

Slika 1: Vpliv biostimulanta na osnovi dihidrokvercetina na skupne fenole in antioksidativno aktivnost plodov žlahtnega jagodnjaka. Različne črke nad stolpcem označujejo statistično značilne razlike pri  $p=0,05$  (Duncanov test)

In conclusion, treatment with the biostimulant based on dihydroquercetin can improve the yield and content of bioactive compounds of strawberry fruit without negatively affecting the quality parameters of the fruit, such as physical traits, color and firmness. It is important to note that the effectiveness of biostimulant preparations based on phenolic compounds may vary depending on depending on the species and specific growing conditions. Therefore, proper application and dosage of these preparations should be carefully studied to achieve optimal results in production.

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