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Article

Biostimulants Improve Bulb Yield, Concomitantly Affecting the Total Phenolics, Flavonoids, and Antioxidant Capacity of Onion (*Allium cepa*)

Đorđe Vojnović [1,*], Ivana Maksimović [1], Aleksandra Tepić Horecki [2], Anita Milić [2], Zdravko Šumić [2], Danijela Žunić [3], Boris Adamović [1,*] and Žarko Ilin [1]

[1] Faculty of Agriculture, University of Novi Sad, Dositej Obradović Square 8, 21000 Novi Sad, Serbia; ivana.maksimovic@polj.uns.ac.rs (I.M.); ilin@polj.uns.ac.rs (Ž.I.)
[2] Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia; tepical@uns.ac.rs (A.T.H.); anitavakula@uns.ac.rs (A.M.); sumic@uns.ac.rs (Z.Š.)
[3] Agricultural Extension Service "PSS Sombor", Staparski put 35, 25000 Sombor, Serbia; psssombor.zunic@gmail.com
* Correspondence: djordje.vojnovic@polj.uns.ac.rs (Đ.V.); borisa@polj.uns.ac.rs (B.A.)

Abstract: In the pursuit of maximizing onion (*Allium cepa*) yield and quality, farmers often face the challenges of unfavorable ecological conditions and inadequate agronomic practices. Therefore, our two-year study investigated the effects of biostimulants (BTs) of plant growth on bulb yield and the bioactive compounds of directly seeded onion. Four treatments were applied: control (C), seaweed extracts (BT1), humic and fulvic acid (BT2), and *Trichoderma* spp. (BT3). The results demonstrated a significant increase in bulb yield with BT1 (↑ 18.7%), BT2 (↑ 18.0%), and BT3 (↑ 24.3%). Intriguingly, all BTs markedly reduced phenolic content across both years. Additionally, BT1 and BT3 elevated flavonoid levels (↑ 16.8% and ↑ 16.7%, respectively), while BT2 decreased them (↓ 24.2%). Notably, in 2021, DPPH, FRAP, and ABTS tests indicated a significant reduction in antioxidant capacity compared to C. Our study underscores the important role of BTs in enhancing yield, influencing secondary metabolites and contributing to environmental sustainability in onion cultivation.

Keywords: onion; bulb; phenolics; flavonoids; antioxidant capacity

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1. Introduction

Onions (*Allium cepa*) are essential components of various dishes and salads, enhancing their taste, aroma, and texture. Furthermore, onions have a rich array of nutrients, containing health-promoting compounds such as phenolics, flavonoids, sugars, and proteins [1,2]. Additionally, due to their high levels of antioxidative compounds, onions are commonly consumed either fresh (e.g., in fast food as salads) or in processed forms (e.g., dried as a seasoning).

Primarily, onion cultivation for fresh consumption is carried out through direct seeding, a method favored for its cost-effectiveness and the higher yields achieved by maximizing plant density per unit area [3,4].

In 2022, onion production accounted for approximately 5,967,491 ha worldwide, yielding a total of about 110.6 million tons of bulbs [5]. In Serbia, onions were cultivated across 4114 ha, with an annual production yield reaching 35,031 tons [5].

In agricultural practices, farmers aim to achieve a high yield of first-class onions to ensure that they attain the highest value in both domestic and global markets [4]. However, a high yield is often accompanied by a lower quality, posing a challenge for consumers, who seek onions with high levels of antioxidative compounds. Therefore, it is essential to strike a balance to ensure that farmers attain a high yield while meeting consumers' expectations for quality.

In recent years, considerable efforts have been dedicated to improving agrotechnical practices in onion production. Novel cultivars have been investigated [6,7], along with plant density [8,9], fertilization [10,11], pesticides [12,13], and irrigation [14,15]. However, these efforts are lacking, as there is an increasing need each year to provide a growing global population with a greater quantity of high-quality onions [16].

One way to advance onion production involves the application of biostimulants (BTs) of plant growth. Laboratory studies have revealed that those substances stimulate various physiological processes in the model plant *Arabidopsis* spp., positively impacting its growth and development [17,18]. Given this assumption, there is a necessity to conduct field experiments with onions using various BTs to ascertain their practicality for adoption by farmers.

Currently, the most commonly used BTs include seaweed extract (SWE) [19], humic substances (HSs) such as humic and fulvic acids (HFAs) [20], and microorganisms such as *Trichoderma* spp. [21]. BTs play a significant role in reducing biotic and abiotic stresses [22], which can lead to a decreased synthesis of plant secondary metabolites [4].

The application of SWE-based BTs was shown to increase the yield of lettuce (*Lactuca sativa*), peppers (*Capsicum annuum*), and tomatoes (*Solanum lycopersicon*) [23,24]. However, in trials with shallots (*Allium cepa Aggregatum* group), no SWE effect on yield was observed [25]. In tomato, SWE enhanced the number and size of xylem and phloem cells in stems, potentially influencing the transport of mineral elements throughout the plant [26,27]. It was also found that SWE improves the expression of genes encoding enzymes involved in carbon fixation during the Calvin cycle [28,29].

In peppers and yarrow (*Achillea millefolium*), the application of HSs increased flavonoid content [30,31], whereas in onions, higher dry matter content was observed after HS treatment [32]. The stimulative effect of HSs is explained by an increase in the activity of proton pumps on the plasma membrane and tonoplast of plant cells [33]. Additionally, HSs enhance the synthesis of phenylalanine and tyrosine ammonia lease, which are involved in the process of phenolic synthesis [34].

In onions from sets, HSs reduced the content of phenolics and antioxidant activity [4]. Conversely, in the case of pepper fruits, there was an increase in phenolic levels [30,35]. This research highlights the complexity of the effects of HS on the distribution of secondary metabolites in different plant organs (i.e., bulbs or fruit) [4].

In vegetable production, fungi from the *Trichoderma* genus can be utilized as BTs [21]. In studies involving lettuce and tomatoes, *Trichoderma* spp. has been shown to increase yields [36,37]. The application of *Trichoderma* spp. also led to increased sugar and protein content in Chinese cabbage (*Brassica campestris* ssp. *chinensis* var. *utilis*) [38], and in lettuce, it enhanced phenolic content [36]. The stimulative effects of *Trichoderma* spp. are primarily attributed to its release of auxins, peptides, and various organic compounds that stimulate plant metabolism [39,40].

Given that onions are a crucial component in numerous dishes and salads worldwide, there is a need to enhance their production to enable farmers to achieve high yields and consumers to enjoy the high nutrition and sensory qualities of onions. Considering the existing research on onions, many studies have explored the impact of BTs on onions produced from seedlings [41–44] and cultivated in pots [45–48]. However, there remains a notable gap in scientific data regarding the impact of BTs on onions directly seeded in the field over a minimum of two years. This knowledge is vital to ensure the practical applicability of results for farmers.

For years, professional farmers have generated substantial profits from onion production. However, in recent years, due to noticeable changes in meteorological conditions, yields of first-class onions have been decreasing. Additionally, markets such as Lidl (Neckarsulm, Germany) [49] or Maxi (Belgrade, Serbia) [50], demand high-quality onion bulbs as consumers seek a higher content of antioxidant compounds in them. Consequently, experts from the Faculty of Agriculture Novi Sad [51], Faculty of Technology Novi Sad [52], and the Agricultural Extension Service Sombor [53], in collaboration with farmers, decided to investigate the effects of new BTs on the yield and quality of onions. Based on the objectives and relevant literature from the Web of Science [54] and Scopus [55] databases, the design of this experiment was defined.

In the light of this context, the principal objective of this study is to evaluate how different BTs impact the yield of first-class bulbs and the content of phytochemicals in them, over a two-year field experiment, in onions produced by direct seeding. Following this objective and based on the reviewed literature, the hypothesis of this study is that the application of various BTs will lead to significant differences in the yield and phytochemical content of onion bulbs.

2. Materials and Methods

2.1. Experimental Design

The field experiment was conducted in Vojvodina province (45.2609° N and 19.8319° E), an agricultural region of Serbia, during 2021 and 2022, using a random block system with three replications. The investigated treatments included: C—Control without BTs; BT1—Agasi® based on SWE: *Laminaria* spp. and *Ecklonia radiata* (Agafert S.R.L. from Bari, Italy); BT2—HumiBlack® based on HFA 15% and K_2O 1.7% (DRN Kimya from Antalya, Turkey); BT3—Tifi® micron powder containing *Trichoderma atroviride* 898G: 2×10^8 UFC/g (Italpollina S.P.A. from Rivoli, Italy).

2.2. Experimental Site

In 2021, seeds were directly sown in the III decade of March following 36 mm of rainfall in the previous decade (Figure 1a). In 2022, there were no significant rainfall events during March, and soil conditions were not wet, leading to earlier sowing in the II decade of March (Figure 1b). The bulb formation in 2021, starting from the III decade of May, occurred at 16.9 °C, while in 2022, bulb formation began in the II decade of May when the temperature was 21.3 °C. These temperatures deviated by −1.1 °C and −4.1 °C from the long-term average, respectively. During bulb formation, air humidity was 69.5% in 2021 and 49.3% in 2022, with both percentages below the long-term average (−0.6% and −19.1%, respectively).

(**a**)

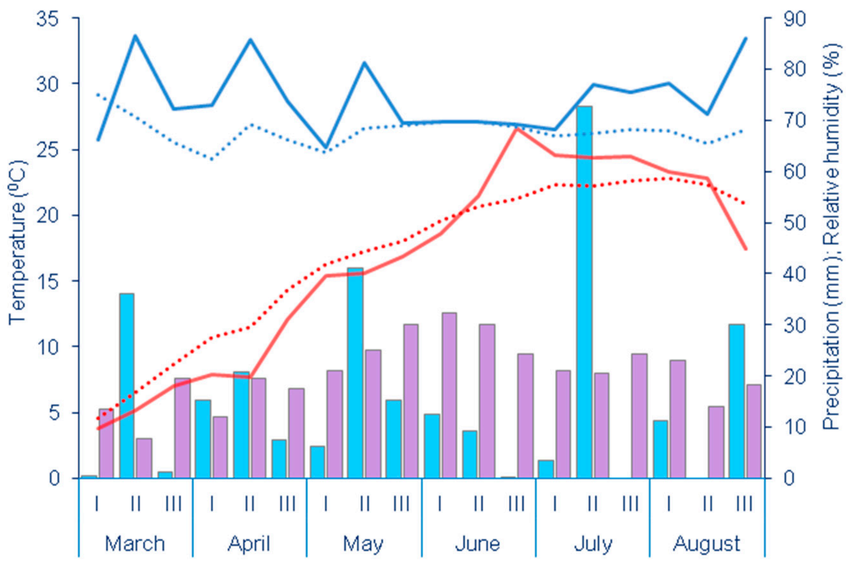

Figure 1. *Cont.*

(**b**)

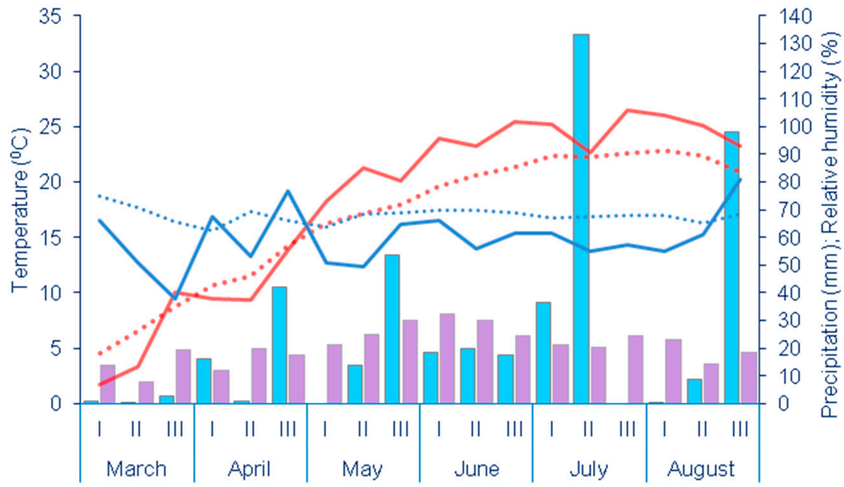

Figure 1. Weather conditions during the experiment in 2021 (**a**) and 2022 (**b**). The average temperature (T)—full red line; the long-term average (1990–2020) (T)—interrupted red line; precipitation (P)—blue bars; long-term average (1990–2020) (P)—purple bars; relative humidity (RH)—full blue line; long-term average (1990–2020) (RH)—interrupted blue line. Roman numerals represent the decade of the month.

Before setting up the experiment, soil samples were analyzed in the laboratory for testing soil, fertilizers, and plant material at the Faculty of Agriculture in Novi Sad. The soil at the experimental site was classified as weakly humic (1.7%), according to Ubavić and Bogdanović [56], as shown in Table 1. The levels of phosphorus (P) and potassium (K), assessed according to Manojlović and Bogdanović [57], indicated moderate levels for P (4.6 mg/100 g) and high levels for K (39.8 mg/100 g). In the following year (2022), the soil exhibited a slightly higher humus level (1.9%), an optimal level of P (6.9 mg/100 g), and a high K (26.6 mg/100 g) content.

Table 1. Agrochemical analysis of soil.

Year	Depth	pH	$CaCO_3$	Humus	N	P	K
		H_2O	(%)	(%)	(%)	mg/100 g Soil	
2021	30 cm	8.1	6.2	1.7	0.08	4.6	39.8
2022		7.8	6.0	1.9	0.13	6.9	26.6

2.3. Agricultural Practices

Agronomic practices during the experiment were described in the study by Vojnović et al. [4]. In brief, the preceding crop was barley (*Hordeum vulgare*) in the first year, while in the second year, the preceding crop was pepper. In the fall, fertilization was performed with the application of 400 kg/ha of combined fertilizer N:P:K 16:16:16 by Yara® (Oslo, Norway). On the same day, plowing was carried out to an average depth of 27 cm using the EurOpal 5 plow by Lemken® (Alpen, Germany), followed by early spring harrowing with a semi-heavy cultivator by Tupanjac® (Futog, Serbia). The first pre-sowing soil preparation was conducted two weeks before sowing using a seedbed cultivator: Swifter SE 10,000 by Bednar® (Praha, Czech Republic). One week later, the second pre-sowing preparation was performed with the same machine. Direct seeding was performed using a pneumatic eight-row seeder SNT-2/3-290 by Agricola Italiana® (Massanzago, Italy). The seeds were sown in double rows with 50 cm spacing between strips, 20 cm between two double rows, 10 cm between two rows in a double row, and 4–6 cm between individual plants. The Elenka F1 cultivar (Cora Seeds®, Cesena, Italy) was used for seeding. Known for its bronze color, pleasant taste, and aroma, it is suitable for both fresh consumption and cooking.

A drip irrigation system was installed using 16 mm diameter drip tapes by Scarabeli Irrigazione® (Bologna, Italy), spaced at 10 cm with a capacity of 10 L/h/m. Irrigation was conducted following the practices from previous research on potatoes and onions [4,58]. Equipment for determining soil moisture content included SKU-6440 sensors by Davis Instruments® (Hayward, CA, USA), connected to a Vantage Pro™ meteorological station from the same manufacturer as the sensors.

BTs were applied twice through the irrigation system at different doses: BT1 at 10 L/ha, BT2 at 50 L/ha, and BT3 at 3 kg/ha [2]. The first application of BTs was administered when the onion reached the second leaf stage (>3 cm), followed by the second application at the third leaf stage (>3 cm).

Onion harvesting took place when 80% of the plants had fallen or when the neck of the bulbs had softened [2]. In 2021, the onions were harvested on August 10, and in 2022, on August 3. The determination of first-class bulb yield (diameter > 40 mm) was conducted under the following regulations: Official Gazzete SFRY 29/79–53/87 [59] and EC No. 1508/2001 [60].

2.4. Laboratory Procedures

2.4.1. Total Phenolic Content

The total phenolic content was spectrophotometrically determined using the Folin–Ciocalteu method [61], with gallic acid as the standard. The total phenolic content is expressed as gallic acid equivalents (mg GAE/100 g DM).

2.4.2. Total Flavonoid Content

The total flavonoid content of the onions was spectrophotometrically determined using the colorimetric method with aluminum chloride [62]. The total flavonoid content is expressed as catechin equivalents (mg CE/100 g DM).

2.4.3. Antioxidant Activity Tests (FRAP, DPPH, and ABTS)

The sample's ability to reduce Fe^{3+} ions (Ferric-Ion-Reducing Antioxidant Power, FRAP) was determined using a modified method based on Benzie and Strain [63]. The results are expressed as milligrams of equivalents of Fe^{2+} ions per 100 g of dry matter (mg Fe^{2+}/100 g DM). The sample's ability to neutralize DPPH radicals was measured using a modified method from Brand-Williams et al. [64]. The results are expressed as milligrams of Trolox equivalents per 100 g of dry matter (mg Trolox/100 g DM). The ABTS free radical scavenging ability of samples was measured using a modified method originally described by Re et al. [65]. The results are expressed as milligrams of Trolox equivalents per 100 g of dry matter (mg Trolox/100 g DM). A UV/VIS spectrophotometer, Jenway® 6300 (Cole—Parmer®, St Neots, UK), was used for absorbance measurements. A detailed description of the conducted methods is presented in the paper by Vakula et al. [66].

2.5. Statistical Data Analysis

To investigate the effect of the year and BTs on onion yield and the content of bioactive compounds, a factorial analysis of variance (ANOVA) was applied. Prior to ANOVA, the normality of distribution was assessed using the Shapiro–Wilk test [67]. The significance of differences between means was tested using the LSD test ($p \leq 0.05$). The relationship between phenolic content and antioxidant activity for each year was individually analyzed through a regression analysis. Statistical analyses were performed using Statistica® 14 by TIBCO Software Inc. (Palo Alto, CA, USA). For a better understanding of the effects of BTs, the treatment averages were subjected to a relative change analysis compared to the control without BT, according to the following formula:

$$\text{Relative change}(\%) = \frac{\text{Treatment}}{\text{Control}} \times 100 - 100$$

3. Results

3.1. Yield of First-Class Onion Bulbs

The application of BTs significantly influenced the yield of first-class onions over the two years of the experiment (Figure 2).

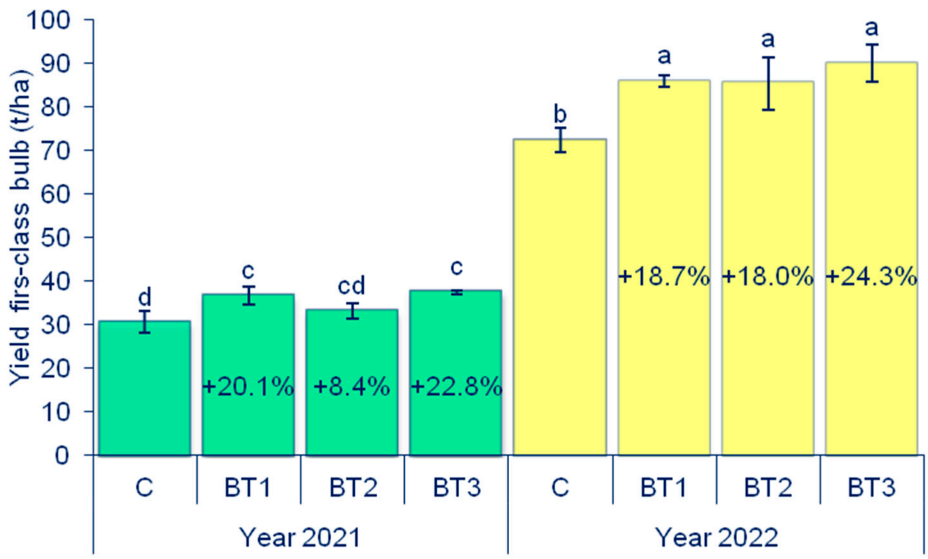

Figure 2. The effect of BTs on the yield of first-class onion bulbs (t/ha) depends on the year. Different letters indicate significant differences ($p \leq 0.05$). Percentages inside the bars represent relative changes compared to C. The line on the bars represents the standard deviation. C—control without BT; BT1—based on SWE; BT2—based on HFA; BT3—based on *Trichoderma* spp.

In 2021, the highest yield was observed in treatment BT3 (37.49 t/ha), representing a 22.8% increase compared to C (30.54 t/ha). Additionally, treatment BT2 (33.10 t/ha) and BT3 (37.49 t/ha) increased the yield of first-class onion bulbs by 20.1% and 8.4%, respectively.

During 2022, the highest yield of first-class onions was recorded in plot BT3 (89.90 t/ha), while the lowest was in plot C (72.95 t/ha), with a statistically significant difference of 18.7%. In the same year, the application of BT2 (85.36 t/ha) and BT3 (89.90 t/ha) led to yield increases of 18.0% and 24.3%, respectively, compared to treatment C.

3.2. Dry Matter Content

In this study, BTs significantly influenced dry matter content in onion bulbs (Table 2).

Table 2. The effect of BTs on dry matter content (%) of onion individually for each year.

Biostimulants	Year 2021	Relative Change (%)	Year 2022	Relative Change (%)
C	9.72 ± 0.12 a	/	10.07 ± 0.03 c	/
BT1	9.56 ± 0.12 a	−1.64	9.73 ± 0.02 d	−3.37
BT2	9.32 ± 0.00 b	−4.11	10.69 ± 0.08 a	+6.15
BT3	8.94 ± 0.05 c	−8.02	10.55 ± 0.25 a	+4.76

Different letters indicate a significant difference ($p \leq 0.05$). Relative change (%) represents the change compared to respective C. Numbers after ± represent the standard deviation. C—control without BT; BT1—based on SWE; BT2—based on HFA; BT3—based on *Trichoderma* spp.

In 2021, the highest dry matter content was recorded in treatment C (9.72%), while the lowest was recorded in BT3 (8.94%), with their difference being statistically significant. In the same year, biostimulants BT1 and BT2 significantly decreased dry matter content compared to C.

In 2022, the highest dry matter content in onions was observed in BT2 (10.69%), significantly exceeding that of C. Additionally, the increase in dry matter was influenced by BT3 (10.55%), while BT1 significantly reduced dry matter content compared to C.

Comparing both years, it is evident that there was a higher dry matter content in 2022 compared to 2021.

3.3. Total Phenolic Content

The application of BTs significantly reduced the phenolic content in onions over the two years of the study (Table 3).

Table 3. The effect of BTs on the total phenolic content (mg/100 g) of onions individually for each year.

Biostimulants	Year 2021	Relative Change (%)	Year 2022	Relative Change (%)
C	1066.06 ± 1.01 a	/	795.28 ± 2.71 e	/
BT1	1047.08 ± 4.60 b	−1.78	744.47 ± 1.03 f	−6.38
BT2	873.27 ± 2.15 c	−18.08	640.70 ± 2.00 h	−19.43
BT3	960.88 ± 4.35 d	−9.86	731.93 ± 4.08 g	−7.96

Different letters indicate a significant difference ($p \leq 0.05$). Relative change (%) represents the change compared to C. Numbers after ± represent the standard deviation. C—control without BT; BT1—based on SWE; BT2—based on HFA; BT3—based on *Trichoderma* spp.

In the first year (2021), the application of BT2 notably decreased the phenol level (18.08%) compared to C, and the difference was significant. Additionally, BT1 and BT3 reduced the phenolic content in bulbs by 1.78% and 9.86%, respectively, compared to C.

In 2022, the highest phenolic content was observed in C (795.28 mg/100 g), while the lowest was in BT2 (640.70 mg/100 g), with a significant difference of 19.43%. The application of BT1 and BT3 reduced the phenolic content by 6.38% and 7.96%, respectively, compared to C.

Observing all treatments across both years, it is evident that the phenolic content was significantly higher in 2021 compared to 2022.

3.4. Flavonoid Content

The flavonoid content in onions varied based on the application of BTs over the two years of the experiment (Table 4).

Table 4. The effect of BTs on the flavonoid content (mg/100 g) of onions individually for each year.

Biostimulants	Year 2021	Relative Change (%)	Year 2022	Relative Change (%)
C	358.07 ± 2.61 b	/	244.15 ± 1.12 e	/
BT1	418.53 ± 10.26 a	+16.88	265.71 ± 3.14 d	+8.83

Table 4. *Cont.*

Biostimulants	Year 2021	Relative Change (%)	Year 2022	Relative Change (%)
BT2	271.31 ± 10.18 d	−24.22	293.99 ± 4.04 c	+20.41
BT3	417.97 ± 2.45 a	+16.72	238.24 ± 4.52 e	−2.42

Different letters indicate a significant difference ($p \leq 0.05$). Relative change (%) represents the change compared to C. Numbers after ± represent the standard deviation. C—control without BT; BT1—based on SWE; BT2—based on HFA; BT3—based on *Trichoderma* spp.

In 2021, the highest flavonoid content was measured in BT1 (418.53 mg/100 g), representing a 16.88% increase compared to C, and the difference was significant. In the same year, BT3 significantly increased flavonoids (+16.72%), while BT2 significantly decreased them (−24.22%) compared to C.

During 2022, the highest flavonoid content was found in plot BT2 (293.99 mg/100 g), indicating a 20.41% increase compared to C (244.15 mg/100 g). Additionally, the application of BT1 (265.71 mg/100 g) significantly increased the flavonoid level by 8.83%, while BT3 (238.24 mg/100 g) reduced flavonoids by 2.42% compared to C.

3.5. Antioxidant Status

In this study, BTs had varied effects on the antioxidant activity of onions, as determined by the DPPH test (Table 5).

Table 5. The effect of BTs on DPPH, FRAP, and ABTS tests (mg TC or Fe2+ mg/100 g) of onion individually for each year.

Year	Biostimulants	DPPH	Relative Change (%)	FRAP	Relative Change (%)	ABTS	Relative Change (%)
2021	C	561.86 ± 1.75 b	/	494.03 ± 0.97 b	/	1489.22 ± 9.53 b	/
	BT1	611.10 ± 4.33 a	+8.76	508.04 ± 1.51 a	+2.83	1508.9 ± 7.60 a	+1.32
	BT2	419.54 ± 4.31 e	−25.33	327.65 ± 2.40 f	−33.67	1245.34 ± 5.42 f	−16.37
	BT3	483.10 ± 2.77 c	−14.01	418.09 ± 2.80 c	−15.37	1333.43 ± 8.29 d	−10.46
2022	C	486.93 ± 1.43 c	/	344.90 ± 2.75 e	/	1284.88 ± 2.67 e	/
	BT1	430.74 ± 2.46 d	−11.53	377.54 ± 1.05 d	+9.46	1367.75 ± 6.54 c	+6.44
	BT2	336.66 ± 3.57 g	−30.86	233.84 ± 1.80 h	−32.20	1010.10 ± 2.05 h	−21.33
	BT3	361.84 ± 2.3 f	−25.68	290.71 ± 0.70 g	−15.71	1150.32 ± 2.98 g	−10.47

Different letters indicate a significant difference ($p \leq 0.05$). Relative change (%) represents the change compared to respective C. Numbers after ± represent the standard deviation. C—control without BT; BT1—based on SWE; BT2—based on HFA; BT3—based on *Trichoderma* spp.

For the DPPH test in 2021, the highest results were observed in BT1 (611.10 mg/100 g), representing an 8.76% increase compared to C (561.86 mg/100 g). In the same year, BT2 (419.54 mg/100 g) and BT3 (483.10 mg/100 g) decreased antioxidant activity by 25.33% and 14.01%, respectively.

In 2022, compared to C (486.93 mg/100 g), the plots where BT1 (430.74 mg/100 g), BT2 (336.66 mg/100 g), and BT3 (361.84 mg/100 g) were applied showed a significant decrease in the DPPH test results by 11.53%, 30.86%, and 25.68%, respectively.

The application of BTs also influenced the results of the FRAP test in onions (Table 5). In 2021, BT1 (508.04 mg/100 g) significantly increased the FRAP test results compared to C (494.03 mg/100 g). However, BT2 (327.65 mg/100 g) and BT3 (418.09 mg/100 g) significantly decreased antioxidant activity by 33.67% and 15.37%.

During 2022, BT1 (377.54 mg/100 g) significantly increased the FRAP test by 9.46% compared to C (344.90 mg/100 g). Similar to the previous year, BT2 (233.84 mg/100 g) and BT3 (290.71 mg/100 g) decreased FRAP test results by 32.20% and 15.71%, respectively.

BTs had varying effects on the antioxidant activity of onions measured using the ABTS test (Table 5). In 2021, treatment BT1 (1508.91 mg/100 g) significantly increased the ABTS test result compared to C (1489.22 mg/100 g). The application of BT2 (1245.34 mg/100 g) and BT3 (1333.43 mg/100 g) significantly decreased antioxidant activity by 16.37% and 10.46%, respectively.

In the following year (2022), treatment BT1 (1367.75 mg/100 g) increased the ABTS test result by 6.44% compared to C (1284.88 mg/100 g). However, BT2 (1010.10 mg/100 g) and BT3 (1150.32 mg/100 g) significantly decreased ABTS test antioxidant activity by 21.33% and 10.47%, respectively.

Figure 3 illustrates the relationship between phenolic content and antioxidant tests (DPPH, FRAP, and ABTS). Throughout 2021 and 2022, a noticeable linear increase in antioxidant activity was observed with the rise in phenolic content in onion bulbs.

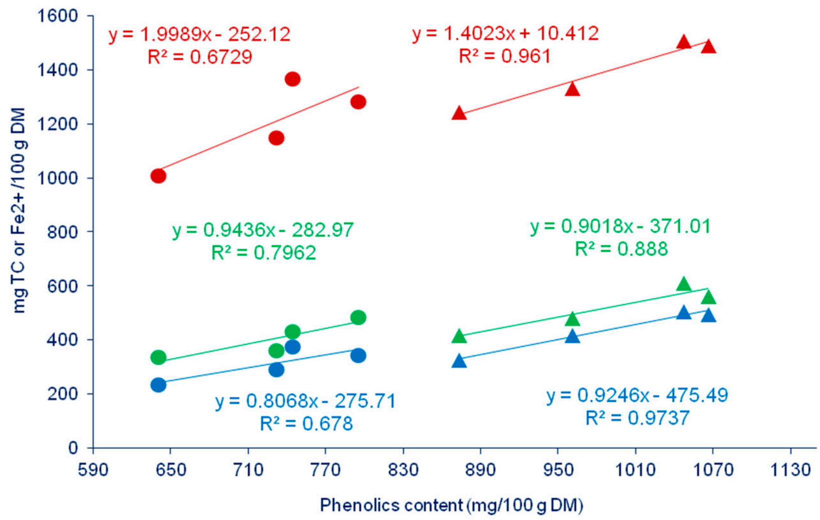

Figure 3. Relationship between phenolics and three antioxidant tests in onions. Circles in the graph represent results from 2022 and triangles from 2021. The red color of symbols and lines represents the ABTS test, the green color represents DPPH, and the blue color represents the FRAP test.

4. Discussion

Onions stand out as one of the most important vegetable plants, playing a significant role in human nutrition. The primary objective of onion production is to achieve a high yield of first-class bulbs, given their premium value in domestic and international markets. According to regulations outlined in the Official Gazette of the SFRY 29/79–53/87 [59] and EC No. 1508/2001 [60], first-class onion bulbs must be healthy, have a diameter of at least 40 mm, and exhibit uniformity in size and color.

In this study, a significantly lower yield was observed in 2021 compared to 2022. According to Lazić et al. [68], onions are sensitive to changes in meteorological conditions during bulb formation. The optimal temperature during this phase, as indicated by the same authors, is 22.0 °C, occurring approximately 60 days after sowing. In line with this, the bulb formation period in 2021 occurred in the third decade of May, with a temperature of 16.9 °C, while in 2022, during this phase, it occurred in the second decade of May, with a recorded temperature of 21.3 °C (Figure 1). According to Lazić et al. [68], humidity above 60% adversely affects bulb formation. In this experiment, in the third decade of May 2021, the registered air humidity was 69.5%, while in 2022, in the second decade of May, it was 49.3% (Figure 1). Additionally, in 2022, higher levels of humus, N, and P were measured compared to the soil in 2021 (Table 1), further explaining the significantly higher yield in 2022. The strong impact of the year of harvest was also noted by Kazimierczak et al. [69], where onion yield in 2012 was 75.8 t/ha, and in the following year, 2013, it decreased to 38.7 t/ha.

In 2021 and 2022, the application of BT1 significantly increased the yield of first-class onions. The stimulating effect of SWE is described by Ali et al. [24], indicating that in peppers and tomatoes, it enhances the expression of genes responsible for the synthesis of auxins, gibberellins, and cytokinins. The effect of SWE can also be explained by an increase in the number and size of xylem and phloem cells in the tomato stem, which can have a favorable impact on the transport of mineral elements through the plant [26,27].

BT2 increased bulb yield, but it was statistically significant compared to C only in 2022. The beneficial effect of HS has been observed in tomatoes [70]. According to Zandonadi et al. [33], the effect of HS can be described by an increase in the activity of proton pumps on the plasma membrane and tonoplast of plant cells.

In vegetable production, fungi from the genus *Trichoderma* spp. can be used as a BT [21]. In 2021 and 2022, BT3 significantly increased the yield of first-class onions. The stimulative effect of *Trichoderma* spp. is mainly based on its ability to release auxins, peptides, and various organic compounds that can enhance plant metabolism [39,40].

Dry matter content is a crucial indicator of the quality of onions. Onions with higher dry matter content exhibit slowed life processes, primarily water loss and respiration. In practical terms, such onions can be stored for an extended period in warehouses. The application of BT1 reduced dry matter content during the two years of the study. This can be explained by the fact that SWE can enhance N uptake in onions [2,41]. An increased N content relative to carbon content in cells signifies a lower fiber (cellulose) content, indicating a higher water content in cells [71].

On plots where BT3 was applied, there was a significant increase in dry matter content over both years. Similarly, in a study with Chinese cabbage (*Brassica campestris* ssp. *chinensis* var. *utilis*), the application of *Trichoderma* spp. significantly increased the content of protein and sugars, which represent components of dry matter [38].

Phenolics are bioactive compounds that significantly contribute to the taste and aroma of onions. The synthesis of secondary metabolites (e.g., phenolics), occurs when the plant is exposed to stress conditions [72]. In this study, the application of all BTs led to a reduction in phenolic content in the bulbs compared to the C. This can be explained by the ability of BTs to mitigate the impact of abiotic stress, resulting in a reduction in the accumulation of secondary metabolites, such as phenolics [4].

Over the two years, the application of BT1 reduced the phenolic content in the onions, which is not in line with the findings of Abbas et al. [41], who discovered an increase in the concentration of phenolics in onions from seedlings using SWE. This can be explained by the fact that onions from seedlings experience stress due to transplanting, compared to direct-seeded onions, increasing phenol synthesis. This is corroborated by Vojnović et al. [4], who state that the phenolic content in onions depends on the production method.

The application of BT2 reduced the phenolic content in onions, a conclusion similar to the findings of Vojnović et al. [71], who reduced phenolics in onion sets using HS. However, in pepper fruit, the use of HS increased phenolics [30,35]. This may be related to the fact that the effect of HS depends on the part of the plant being studied [4].

In a study on tomatoes, the application of *Trichoderma* spp. reduced the phenolic content in the fruit [40]. Similarly, the application of BT3 reduced phenolic content in onion bulbs in this study.

Flavonoids are essential bioactive compounds that significantly impact the taste of onions and have numerous health benefits due to their potent antioxidant properties [2,73]. Antioxidants are essential for protecting the human body from harmful radicals, thereby reducing the risk of various diseases [74]. As critical secondary metabolites, flavonoids play a significant role in plant defense against different biotic and abiotic stresses [74]. The application of BT1 significantly increased flavonoid content in 2021 and 2022. Similar results have been documented in onions, carrots (*Daucus carota*), and beans (*Phaseolus vulgaris*) following SWE application [4,75,76]. This can be explained by the fact that SWE enhances the expression of genes involved in the synthesis of enzymes participating in the phenylpropanoid pathway, a process essential for flavonoid synthesis [18].

On plots where BT2 was used in 2022, there was a 20.41% increase in flavonoid levels. This can be attributed to the fact that HS improves the synthesis of phenylalanine and tyrosine ammonia lyase, enzymes involved in the flavonoid synthesis process [34]. The application of HS on plots with pepper and yarrow (*Achillea millefolium*) has been shown to increase flavonoid content [30,31].

In a study by Vojnović et al. [4], the application of *Trichoderma* spp. reduced flavonoid content in directly seeded onions fertilized with 150 kg N/ha. However, in this study, during 2021, the use of the BT3 increased flavonoid levels by 16.72%. This aligns with Vukelić et al. [40], who recorded increased flavonoid content in tomatoes with *Trichoderma* spp. application. Consistent with this, recent findings indicate that *Trichoderma* spp. activates the phenylpropanoid pathway in flavonoid synthesis [77,78].

Antioxidant activity in vegetables plays a crucial role in reducing the risk of chronic diseases, including cardiovascular diseases, anemia, and cancer [74,79]. According Zehiroglu et al. [79] and Arias et al. [80], antioxidants protect human cells from damage caused by free radicals and reactive oxygen species. In 2021, on plots where BT1 was applied, the antioxidant activity of onions significantly increased, as confirmed by DPPH, ABTS, and FRAP tests. The increased flavonoid content can explain this, as flavonoids exhibit strong antioxidant properties [73].

BT2 and BT3 reduced the antioxidant activity of onions, a trend confirmed over the two years of the study. This reduction could be ascribed to the decreased phenolic content in these treatments, as phenolics have significant antioxidant properties. This is further supported by a linear relationship between phenolics and antioxidants (DPPH, FRAP, and ABTS), which was found in this study (Figure 3). A similar correlation was also observed by Vojnović et al. [2].

5. Conclusions

The application of BT1 and BT3 significantly increased bulb yield compared to the respective C across both years of the experiment.

On plots treated with BT1, BT2, and BT3, phenolic content was significantly reduced. The application of BT1 significantly increased flavonoids and antioxidant activity (FRAP and ABTS) in both years.

Based on the findings, the recommendation for farmers would be to use BT1 and BT3 to achieve high bulb yields in the production of onions from direct seed sowing, while BT1 is recommended for enhancing antioxidant capacity. Additionally, the application of BTs has environmental significance as they are made from natural substances that cannot harm the environment.

Our study confirms that various BTs significantly influence bulb yield and phytochemical content in onions grown through direct seeding. This supports our initial hypothesis. Further research could explore the impact of BTs on onions under reduced agricultural practices.

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