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The Biostimulation Activity of Two Novel Benzothiadiazole Derivatives in the Tomato Cultivation

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Abstract: Biostimulants are gaining more and more attention in modern agriculture. As follows from the definition, their role is aimed at influencing the plant's metabolism, which results in increasing the quantitative and qualitative parameters describing the yield. Significant attention should be paid to biostimulants increasing the content of health-promoting substances contained in plants. Treatments with biostimulants should be properly incorporated into existing plant protection schemes, which, of course, requires detailed research in this area. However, reliable research on active substances contained in biostimulants should be made first, and the activity of a given biostimulant must be proven. This work presents the results of a field experiment in tomato cultivation for two new active substances belonging to the group of benzothiadiazoles. The results indicate a positive effect on plant yield and, above all, on the lycopene content in tomato fruits. Increasing the lycopene content in fruit is of key significance, as it opens up opportunities for these active substances to be incorporated into new plant protection programs.

Keywords: organic salts; benzotiadiazoles; biostimulation; SAR induction; growth immunity tradeoff; tomato; lycopene; sustainable agriculture

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1. Introduction

The possibility of ensuring increased efficiency of agricultural production is of key significance due to the growing demand for food related to the ever-increasing world population. To achieve this goal, more and more agrochemicals are used, including plant protection products (PPPs), which are to provide effective protection of plants against pathogens. It is estimated that losses caused by pathogens can lead to up to 70% reduction in crop yields worldwide. However, extensive use of pesticides causes a threat not only to the environment but also to humans [1,2]. The European Union, regarded as the leader in the legislation on ecological development and natural environment protection, has recognised the need for sustainable development, including curbed use of pesticides to reduce the threat related to their activity and impact on human health and the environment and incentivise the measures aimed at integrated plant protection and use of alternative approaches and techniques. The desire to reach these goals is expressed in the "Farm to Fork Strategy", which specifies the requirements for a 50% reduction in the use of pesticides by 2030. Moreover, the number of active substances of PPPs that are authorised to be used will decrease as a result of the implementation of Regulation 2015/408/EC [3]. The first to eliminate are often the substances most effective against pathogens but also the most toxic to the natural environment [4]. Implications of the above-mentioned activities have led to the stimulation of the need to search for new, effective and sustainable methods of plant protection with the use of novel active substances satisfying the legal requirements and societal expectations. In a broader sense, the implications of indicated activities are related to the general need to increase sustainability [5–7]

An alternative and sustainable approach that may serve as a response to indicated need is SAR inducers, which are still not widely used in agricultural practice. As for their mechanism of action, SAR inducers trigger the induction of Systemic Acquired Resistance phenomena, which constitute the natural defence mechanism of plants. This phenomenon is related to the increased expression of pathogenic-related genes (PR genes) in the plant's tissue as a response to the action of the pathogen [8]. This mechanism can be triggered not only by the action of various pathogens but also by the application of SAR inducers of both natural and chemical origin [9,10]. Regardless of the elicitor, the process is mediated by salicylic acid (SA).

There are several science-based reasons why SAR inducers are not yet widely used in agriculture. One of them is related to the risk of occurrence of another phenomenon that is strongly linked with SAR induction, known as the growth–immunity trade-off [11–13]. The result of this process, which is also mediated by SA, might be manifested in a reduction in plant yield caused by the change in plant resource allocation related to SAR induction [14]. Therefore, attention should be paid to the appropriate application of tested SAR inducers, meaning their use in proper concentration, amount of treatments performed during the vegetation period and interval between application.

The most studied SAR inducer is benzo[1.2.3]thiadiazole-7-carboxylic acid, *S*-methyl ester (BTH), an active substance of products Actigard 50 WG and Bion 50 WG by Syngenta company (Basel, Switzerland) [15,16]. This substance is composed of a thiadiazole ring conjugated with a benzene ring that is substituted by a carboxyl functional group on the C-7 carbon atom. As the structure has a strong aromatic nonpolar character, its solubility in water is poor (7 mg/L). Our previous studies have been focused on the synthesis of novel, both neutral and ionic, derivatives of BTH, with the aim to increase the water solubility of the compounds and maintain or improve their biological function. Compounds with the highest SAR induction efficiency manifested as the inhibition of tobacco mosaic virus in tobacco plants were then subjected to detailed molecular study [17]. Plants treated with *N*-methoxy-*N*-methylbenzo[1.2.3]thiadiazole-7-carboxamide (BTHWA) and choline benzo[1.2.3]thiadiazol-7-carboxylate (BTHChol) showed a significant increase in the expression level of SAR marker genes such as PAL, NPR1, and PR-1b. Moreover, the analysis of the viral RNA accumulation level revealed that viral replication in plants treated with these two compounds was lower compared not only to plants of untreated control but also to plants treated with parent compound BTH. Hence, further agronomic research was carried out on these two novel active substances. In other agronomic studies, we investigated the effectiveness of the tested active substances in providing effective protection against pathogens. But also, we wanted to check whether the growth-immunity trade-off phenomenon occurs, and if so, to what extent. The results from previous work showed that in the case of our substances, the growth immunity tradeoff did not occur with 4 applications of the BTHWA substance on tulip plants [18]. Moreover, the effect of biostimulation on the growth of tulip plants treated with BTHWA was observed, both in terms of experiments with and without pathogen presence in the substrate. The results regarding such activity of the tested substances are confirmed in our other studies, which have not yet been published.

As for the general aim of this work, we originally wanted to check whether it is possible to obtain growth immunity trade-off when applying BTHWA and BTHCholine in a total of 6 or 9 times in a program combined with a standard protection program (later referred to as SFP). Moreover, two different doses of both substances were tested, one of them being the standard dose established in previous studies, while the second dose was twice as high as the one optimally used.

Firstly, the methodology section related to the description of tested substances, as well as the experimental design and scope of analyses performed in this study, is presented.

Secondly, the results describing the quantitative and qualitative parameters of yield that were obtained after statistical analysis of data collected are shown. This is followed by a discussion of the results. The discussion concerns both the results presented in this work in a narrow context as well as in the broader context that is related to the possible impact of introducing testes substances to agricultural practice and future plans. Finally, the conclusions section is provided.

2. Materials and Methods

This study presents the influence of tested variants of treatments with BTHWA and BTHCholine on qualitative and quantitative parameters of tomato yield. The methodological section consists of a description of the tested active substances and assumptions regarding the technology of their application. Then, data regarding the field experiment are presented along with a description of the experimental sites. Since the occurrence of growth-immunity tradeoff was expected, it was planned to check whether it would be manifested in the total yield, fruit size, lycopene content and total soluble sugar (TSS) content. Finally, a description of performed statistical analysis is provided.

2.1. Tested Substances

The substances studied were both neutral derivatives of benzotiadiazole named *N*-methoxy-*N*-methylbenzo(1.2.3)thiadiazole-7-carboxamide (BTHWA) and ionic derivative of benzotiadiazole named choline benzo[1.2.3]thiadiazol-7-carboxylate (BTHCholine). Both derivatives were obtained by our group with a purity of 99.9%, and the water solubility of both compounds is improved compared to the solubility of the parent compound BTH. BTHWA substance is soluble in water in the approximate amount of 20 mg/L, while BTHCholine, due to its ionic nature, is soluble in water in an amount higher than 10 g/L. Thus, for the BTHWA, the SC type of formulation was prepared. The amount of BTHWA contained in 1 L of formulation was equal to 10 g. As for BTHCholine, 10 g of active substance was soluble in water. Such starting solutions were used to prepare the spray liquid.

Both substances were applied in two-week intervals, in two different manners, i.e., in a total of either 6 or 9 treatments during the tomato growing season, starting from its beginning. Moreover, both substances were applied in two different concentrations, one of them being the optimal concentration, i.e., 20 mg/L, and the other was a concentration twice as high, applied with the intention to provoke a potential growth-immunity tradeoff phenomenon. Overall, this experimental setup resulted in the creation of 4 treatment variants for each substance, marked 6×20, 9×20, 6×40, and 9×40, respectively, where the first number indicates the number of treatments and the second number indicates the concentration of a given substance.

2.2. Field Experiment

A field experiment was conducted at the private experimental field near Kotlin in Poland (51°57′51.7′′ N 17°41′50.2′′ E) from April to September of 2021 and 2022 on the cultivation of tomato var. Dyno F1. The meteorological data during the experimental period were obtained from the weather station of Kotlin and are presented in Figure 1. The experiment was carried out on sandy loam soil. Before the start of each experiment, the soil was analysed, and its characteristics are provided in Table 1.

For the experiment, a system of randomised plots was established with 4 plots per each variant of treatment. The area of each plot was equal to 9.1 m^2, and 19 plants with spacing 0.70 m $\times$ 0.65 m were grown in this area. This accounts for the stocking of 21,970 plants per hectare. The already prepared tomato seedlings were planted in May.

Plants of all variants of treatment were equally protected with standard plant protection products (later referred to as SFP), the list of which is provided in Table S1, ESI. All of the treatments, including treatments with both tested substances, were performed with the "Stihl SR 420" knapsack-motor sprayer with a tank capacity of 14 L with a pneumatic sprayer, using 500 L of working fluid per 1 ha.

Fruits were harvested manually during a total of 5 harvests in each of the experimental years. The first harvest took place on 26 August 2021 and 28 August 2022, respectively. Fruits that met ripening criteria were harvested at weekly intervals. Harvested fruits were first divided into marketable and non-marketable yields. Results were extrapolated, based on the number of plants, to calculate values of these parameters per hectare.

Fruits classified as marketable yield were free from mechanical damage and visible symptoms of any infections, the impact of which in the experiment was neglected by the use of a standard protection program in each of the experimental variants of treatment. Fruits of marketable yield were subsequently classified in terms of their diameter. The classification was based on three diameter classes: fruits with a diameter of less than 3.5 cm, fruits with a diameter between 3.5 cm and 4.5 cm, and fruits with a diameter of more than 4.5 cm. During this assessment, the fruits were counted, and then all fruits of a given diameter class were weighed together. Then, representative samples of fruits were collected for further analysis.

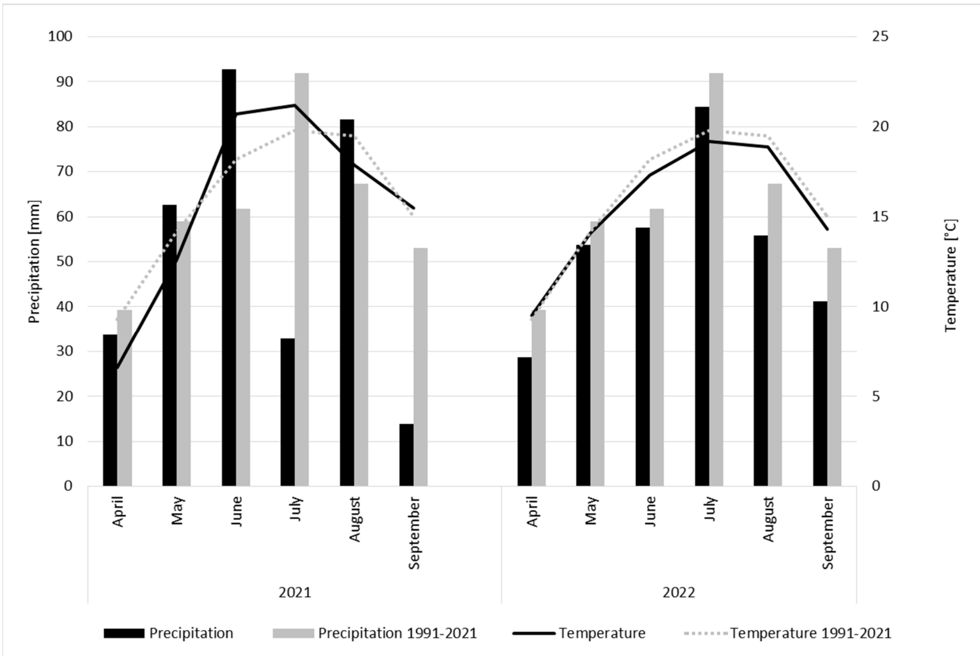

Figure 1. Weather conditions (average temperature and precipitation) at experimental location Kotlin in years 2021–2022.

Table 1. Description of experimental sites.

Year of Experiment	Soil Characteristics	pH	$N\text{-}NO_3$	P	K	Ca	Mg	Cl
			[mg/dm^3 of Soil]					
2021	sandy loam	7.4	9.7	94.6	171.6	942.8	182.6	4.2
2022	sandy loam	7.5	9.4	95.2	186.5	956.1	174.9	4.8

2.3. Content of Bioactive Compounds in Tomato Fruit

Total soluble solids content was measured with an Atago Refractometer RX-7000CX (Atago, Saitamam, Japan) at a standardised temperature (20 °C) in fruit juices obtained by manually pressing 200 g of fruits from each replication. Two samples from each replication were analysed, which gives a total of 8 samples per variant of treatment. Results are expressed in °Brix.

The quantification of lycopene in the fruit utilised the Ranganna method [19]. Specifically, approximately 1 g of the fruit pulp was blended with 5 mL of acetone and then poured into a separation funnel. Following this, the mixture was enriched with 20 mL of petroleum ether and 20 mL of 5% sodium sulphate, ensuring thorough mixing. Post-incubation, the upper layer of petroleum ether was isolated to collect the aqueous phase beneath it. Subsequently, an additional 20 mL of petroleum ether was introduced to further extract lycopene. Around 10 g of anhydrous sodium sulphate was then mixed into the petroleum ether solution and left to stand for 20 min. The resulting petroleum ether solution was gathered in a 25 mL volumetric flask, which was then filled to the mark with petroleum ether to adjust the volume to 25 mL. The absorbance of the sample was read at 503 nm using a spectrophotometer Spectroquant Pharo 300 (Merck, Darmstadt, Germany), and it was expressed as mg 100 g^{-1}. Two samples from each replication were analysed, which gives a total of 8 samples per variant of treatment.

2.4. Statistical Analysis

The data were analysed from 4 blocks for each variant of treatment. Statistical assumptions related to the normality and homogeneity of variances were checked. All recorded and calculated data were evaluated by analysis of variance (ANOVA), and the mean differences were compared by post hoc test at a $p < 0.05$ level, according to Tukey's HSD. Statistical analyses were performed using the software of OriginLab 2022 (OriginLab Corp., Northampton, MA, USA).

3. Results

3.1. Weather Conditions

Figure 1 presents the meteorological data recorded during all experimental periods. The warmest month of 2021 was July. The average daily air temperature recorded in this month was 21.2 °C, which was 2.5 °C higher than the average for 1991–2021. In 2022, the highest average daily temperature was recorded in August (19.2 °C). It was lower than the August average daily temperature observed in the multi-year period by 0.6 °C.

As for the total precipitation, it was higher in 2022 compared to the total precipitation of 2021. It was equal to 321.4 mm and was 45.1 mm lower than the average precipitation noted during the same period of the year 1991–2021. In 2021, total precipitation was also lower compared to the average for 1991–2021. The difference in total precipitation between comparing periods was equal to 49.5 mm.

3.2. Experiment 2021

3.2.1. General Yield

The marketable yield of plants treated with all tested variants of treatment with BTHWA was significantly higher compared to that of SFP (Table 2). The same applies to the non-marketable yield of plants traded according to variants BTHWA 6 × 20 and BTHWA 6 × 40. Significant differences for this parameter were not observed for variants BTHWA 9 × 20 and BTHWA 9 × 40. Of note, the ratio of non-marketable to marketable yield for all tested variants of treatment with BTHWA was lower compared to this of SFP. The total yield of SFP-treated plants was significantly the lowest.

Similar trends are observed for variants consisting of treatment with BTHCholine. Treatment according to variants BTHCholine 6 × 20 and BTHCholine 6 × 40 resulted in significantly higher marketable yield compared to this of SFP (Table 2). The same applies to the non-marketable yield and total yield. Differences for indicated parameters are also observed for variant BTHCholine 9 × 20. However, they are not statistically proven. As in the case of plants treated with BTWHA, the ratio of non-marketable to marketable yield for all tested variants of treatment with BTHCholine is also lower compared to this of SFP.

Table 2. Quantitative parameters describing the yield as a result of treatment according to tested variants.

	Marketable Yield [t/ha]	Non-Marketable Yield [t/ha]	Total Yield [t/ha]	Ratio of Non-Marketable to Marketable Yield [%]
SFP	74.05 ± 2.03 a	8.30 ± 0.23 a	82.35 ± 2.26 a	11.2
BTHWA 6 × 20	88.78 ± 1.18 d	9.59 ± 0.13 c	98.37 ± 1.31 d	10.79
BTHWA 9 × 20	83.09 ± 2.67 bc	8.55 ± 0.28 ab	91.64 ± 2.95 bc	10.29
BTHWA 6 × 40	84.93 ± 1.3 cd	9.03 ± 0.14 b	93.96 ± 1.43 cd	10.63
BTHWA 9 × 40	79.98 ± 1.59 b	8.09 ± 0.15 a	88.07 ± 1.74 b	10.14
SFP	74.05 ± 2.03 a	8.30 ± 0.23 a	82.35 ± 2.26 a	11.2
BTHCholine 6 × 20	83.65 ± 3.04 b	8.97 ± 0.33 c	92.62 ± 3.37 b	10.72
BTHCholine 9 × 20	78.4 ± 2.30 ab	8.09 ± 0.15 a	86.49 ± 2.45 ab	10.32
BTHCholine 6 × 40	82.89 ± 1.99 b	8.76 ± 0.21 bc	91.65 ± 2.2 b	10.57
BTHCholine 9 × 40	75.86 ± 1.99 a	7.74 ± 0.2 a	83.60 ± 2.19 a	10.21

Symbols for the table: for each active substance separately, within the columns, mean values marked with lowercase letters differ significantly depending on the variant of treatments at $p = 0.05$ according to Tukey's HSD, while mean values are not marked with a letter if differences are not significant. SFP—plants treated with standard fungicide treatment; BTHWA 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHWA 9 × 20—plants treated with BTHWA 20 mg/L in total of 9 times; BTHWA 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHWA 9 × 40—plants treated with BTHWA 40 mg/L in total of 9 times; BTHCholine 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHCholine 9 × 20—plants treated with BTHCholine 20 mg/L in total of 9 times; BTHCholine 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHCholine 9 × 40—plants treated with BTHCholine 40 mg/L in total of 9 times. The full list of treatments is shown in Table S1 in the ESI.

3.2.2. Tomato Yield Structure

Significant changes are in terms of the distribution of the number of fruits in each of the diameter classes (Table 3). For both active substances tested, these differences are mainly manifested in the number of fruits with diameters of 3.5–4 cm and 4.0–4.5 cm. For all tested variants assuming treatment with either BTHWA or BTHCholine, the highest amount of fruits having a diameter of 3.5–4 cm was observed for fruits of SFP. As for the fruits of a diameter of 4.0–4.5 cm, the lowest amount was observed for fruits of SFP, but the difference compared to all tested variants of treatment is significant only for a variant of BTHWA 6 × 20, BTHWA 6 × 40, BTHCholine 6 × 20 and BTHCholine 9 × 20. As regards fruits with the highest diameter, a significant increase compared to fruits of SFP was observed only for BTHWA 6 × 20 and BTHCholine 6 × 40.

Of note, despite these differences in terms of the distribution of the number of fruits in each of the diameter classes, no variability was noted in terms of the total number of fruits from the plots.

The total mass of the fruits was also affected by the application of tested substances (Table 4). For both active substances tested, the total mass of fruits with a diameter of 3.5–4 cm was the highest for the plants of SPF. However, the difference between particular treatment variants is not statistically significant for all of them. Overall, the decreased mass of fruits for tested variants of treatment for both active substances is related to a significant decrease in the number of fruits (Table 3). As regards fruits with a diameter of 4.0–4.5 cm, a significant increase in the total mass of fruits is observed for variants of treatment with both testes substances compared to plants treated according to SFP, except from variant BTHCholine 6 × 40. A similar trend is observed for the fruits with the highest diameter. The increase in the total mass of fruits compared to the SFP variant is not statistically confirmed only in the case of variants BTHWA 9 × 40, BTHCholine 9 × 20 and 2 BTHCholine 9 × 40.

Of note, significant changes were observed for the sum of the total mass of fruits for all three diameter classes. For the BTHWA, a significant increase compared to SFP was observed for all tested variants of treatment, with BTHWA 6 × 20 having the highest sum of total mass. For the BTHCholine, a significant increase was only observed for variants BTHCholine 6 × 20 and BTHCholine 6 × 40.

Table 3. The number of fruits of different diameters as a result of treatment according to tested variants.

	The Number of Fruits [pcs/plot]			
	Diameter 3.5–4 cm	**Diameter 4.0–4.5 cm**	**Diameter >4.5 cm**	**Sum of All Fruits**
SFP	249 ± 12.68 d	294 ± 8.62 a	418 ± 6.48 a	961 ± 10.85
BTHWA 6 × 20	168 ± 8.87 a	343 ± 9.49 c	448 ± 20.14 b	959 ± 36.75
BTHWA 9 × 20	197 ± 4.64 bc	314 ± 10.68 ab	429 ± 14.08 ab	940 ± 25.17
BTHWA 6 × 40	189 ± 15.08 ab	324 ± 14.29 bc	439 ± 18.73 ab	952 ± 22.56
BTHWA 9 × 40	220 ± 10.63 c	315 ± 7.46 ab	428 ± 11.61 ab	963 ± 10.93
SFP	249 ± 18.68 c	294 ± 8.62 a	418 ± 6.48 a	961 ± 10.6
BTHCholine 6 × 20	159 ± 12.54 a	350 ± 9.14 c	419 ± 12.78 a	928 ± 34.34
BTHCholine 9 × 20	200 ± 7.27 b	322 ± 9.81 b	394 ± 13.2 a	916 ± 29.60
BTHCholine 6 × 40	168 ± 9.42 a	291 ± 11.78 a	447 ± 9.78 b	906 ± 29.95
BTHCholine 9 × 40	210 ± 5.51 b	301 ± 10.9 ab	410 ± 12.92 a	921 ± 34.4

Symbols for the table: within the columns, mean values marked with lowercase letters differ significantly depending on the variant of treatments at p = 0.05 according to Tukey's HSD, while mean values are not marked with a letter if differences are not significant. SFP—plants treated with standard fungicide treatment; BTHWA 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHWA 9 × 20—plants treated with BTHWA 20 mg/L in total of 9 times; BTHWA 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHWA 9 × 40—plants treated with BTHWA 40 mg/L in total of 9 times; BTHCholine 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHCholine 9 × 20—plants treated with BTHCholine 20 mg/L in total of 9 times; BTHCholine 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHCholine 9 × 40—plants treated with BTHCholine 40 mg/L in total of 9 times The full list of treatments is shown in Table S1 in the ESI.

Table 4. The total mass of fruits of different diameters as a result of treatment according to tested variants.

	Total Mass of Fruits [kg/plot]			
	Diameter 3.5–4 cm	**Diameter 4.0–4.5 cm**	**Diameter >4.5 cm**	**Sum**
SFP	8.04 ± 0.42 b	17.18 ± 0.77 a	38.82 ± 2.5 a	64.04 ± 2.03 a
BTHWA 6 × 20	6.00 ± 0.52 a	21.41 ± 0.57 c	49.36 ± 0.74 c	76.77 ± 1.18 d
BTHWA 9 × 20	7.37 ± 0.44 b	19.52 ± 0.68 d	44.97 ± 2.05 b	71.86 ± 2.67 bc
BTHWA 6 × 40	7.33 ± 0.24 b	21.30 ± 0.75 c	44.81 ± 1.1 b	73.44 ± 1.29 cd
BTHWA 9 × 40	7.66 ± 0.66 b	19.67 ± 0.84 b	41.84 ± 1.09 ab	69.17 ± 1.58 b
SFP	8.04 ± 0.42 c	17.18 ± 0.77 a	38.82 ± 2.5 a	64.04 ± 2.03 a
BTHCholine 6 × 20	5.29 ± 0.29 a	21.27 ± 0.65 d	45.77 ± 2.17 b	72.33 ± 3.04 b
BTHCholine 9 × 20	6.83 ± 0.16 b	19.68 ± 0.36 c	41.30 ± 1.81 ab	67.81 ± 2.29 ab
BTHCholine 6 × 40	5.74 ± 0.37 a	17.69 ± 0.64 ab	48.26 ± 0.98 b	71.69 ± 1.99 b
BTHCholine 9 × 40	7.37 ± 0.2 bc	18.68 ± 0.61 bc	39.97 ± 1.29 a	66.02 ± 1.38 a

Symbols for the table: within the columns, mean values marked with lowercase letters differ significantly depending on the variant of treatments at p = 0.05 according to Tukey's HSD, while mean values are not marked with a letter if differences are not significant. SFP—plants treated with standard fungicide treatment; BTHWA 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHWA 9 × 20—plants treated with BTHWA 20 mg/L in total of 9 times; BTHWA 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHWA 9 × 40—plants treated with BTHWA 40 mg/L in total of 9 times; BTHCholine 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHCholine 9 × 20—plants treated with BTHCholine 20 mg/L in total of 9 times; BTHCholine 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHCholine 9 × 40—plants treated with BTHCholine 40 mg/L in total of 9 times. The full list of treatments is shown in Table S1 in the ESI.

An increase in the average mass of fruit resulting from the application of tested variants of treatment was observed for both tested substances (Table 5). A significant increase in the average mass of fruit compared to fruits of SFP was observed for tomato fruits of every diameter class treated with all variants of treatment with BTHWA, except from the variant BTHWA 9 × 40. In general, results for variants of treatment with BTHCholine follow the same trend, although not all differences are statistically proven. For fruits with the highest diameter, the use of all treatment variants resulted in a significant increase in the average fruit weight compared to SFP.

Table 5. Average mass of fruit of different diameters as a result of treatment according to tested variants.

	Average Mass of Fruit [g]			
	Diameter 3.5–4 cm	**Diameter 4.0–4.5 cm**	**Diameter >4.5 cm**	**Weighted Average of Fruit [g]**
SFP	32.27 ± 0.45 a	58.38 ± 2.82 a	93.04 ± 3.01 a	66.66 ± 1.75 a
BTHWA 6 × 20	35.75 ± 1.01 bc	62.46 ± 1.03 ab	109.70 ± 3.84 c	79.90 ± 1.92 d
BTHWA 9 × 20	37.51 ± 1.92 bc	62.21 ± 2.1 ab	104.64 ± 1.54 bc	76.43 ± 0.86 c
BTHWA 6 × 40	38.89 ± 1.94 c	65.77 ± 1.63 b	102 ± 4.08 b	77.10 ± 1.95 cd
BTHWA 9 × 40	34.72 ± 1.78 ab	65.40 ± 1.86 ab	97.72 ± 3.69 ab	71.75 ± 0.95 b
SFP	32.27 ± 0.45 a	58.38 ± 2.82 a	93.04 ± 3.01 a	66.66 ± 1.75 a
BTHCholine 6 × 20	33.30 ± 1.3 ab	60.74 ± 0.7 ab	109.2 ± 2.21 d	77.92 ± 0.45 d
BTHCholine 9 × 20	34.05 ± 0.54 b	61.16 ± 0.77 ab	104.73 ± 1.29 c	73.97 ± 0.2 c
BTHCholine 6 × 40	34.15 ± 0.89 b	60.73 ± 0.33 ab	108.03 ± 0.87 cd	79.13 ± 0.48 d
BTHCholine 9 × 40	34.89 ± 0.68 b	62.09 ± 3.6 b	97.39 ± 1.35 b	71.60 ± 0.61 b

Symbols for the table: within the columns, mean values marked with lowercase letters differ significantly depending on the variant of treatments at p = 0.05 according to Tukey's HSD, while mean values are not marked with a letter if differences are not significant. SFP—plants treated with standard fungicide treatment; BTHWA 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHWA 9 × 20—plants treated with BTHWA 20 mg/L in total of 9 times; BTHWA 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHWA 9 × 40—plants treated with BTHWA 40 mg/L in total of 9 times; BTHCholine 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHCholine 9 × 20—plants treated with BTHCholine 20 mg/L in total of 9 times; BTHCholine 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHCholine 9 × 40—plants treated with BTHCholine 40 mg/L in total of 9 times. The full list of treatments is shown in Table S1 in the ESI.

Of note, weighted average values for the average fruit weight indicate a significant increase compared to the SFP variant for all tested variants for treatments for both substances. The highest increase in the value of this parameter was observed for variants assuming a total of 6 treatments.

3.2.3. Bioactive Substances

Tested variants of treatment also affected the content of lycopene and total soluble solids in fruits (Table 6). As for the content of lycopene, a significant increase compared to fruits of SFP is observed for all variants of treatment with both substances; however, for the variants BTHCholine 6 × 40 and BTHCholine 9 × 40, the difference is not statistically proven. As for the content of TSS, a significant increase compared to fruits of SFP is observed for all variants of treatment with both substances; however, for the variants BTHWA 6 × 40, BTHWA 9 × 40 and BTHCholine 9 × 40, the difference is not statistically proven.

Table 6. The content of lycopene and total soluble sugar (TSS) in tomato fruits was treated according to tested variants of treatment.

	Lycopene [mg/100 g/DW]	**TSS [°Brix]**
SFP	3.97 ± 0.1 a	6.92 ± 0.08 a
BTHWA 6 × 20	4.65 ± 0.09 b	7.56 ± 0.06 b
BTHWA 9 × 20	4.64 ± 0.078 b	7.45 ± 0.1 b
BTHWA 6 × 40	4.36 ± 0.07 b	7.365 ± 0.095 ab
BTHWA 9 × 40	4.375 ± 0.1 b	7.14 ± 0.09 ab

Table 6. *Cont.*

	Lycopene [mg/100 g/DW]	TSS [°Brix]
SFP	3.97 ± 0.1 a	6.92 ± 0.08 a
BTHCholine 6 × 20	4.65 ± 0.067 b	7.5 ± 0.08 b
BTHCholine 9 × 20	4.55 ± 0.09 b	7.43 ± 0.06 b
BTHCholine 6 × 40	4.17 ± 0.13 ab	7.41 ± 0.075 b
BTHCholine 9 × 40	4.21 ± 0.18 ab	7.23 ± 0.115 ab

Symbols for the table: for each active substance separately, within the columns, mean values marked with lowercase letters differ significantly depending on the variant of treatments at p = 0.05 according to Tukey's HSD, while mean values are not marked with a letter if differences are not significant. SFP—plants treated with standard fungicide treatment; BTHWA 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHWA 9 × 20—plants treated with BTHWA 20 mg/L in total of 9 times; BTHWA 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHWA 9 × 40—plants treated with BTHWA 40 mg/L in total of 9 times; BTHCholine 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHCholine 9 × 20—plants treated with BTHCholine 20 mg/L in total of 9 times; BTHCholine 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHCholine 9 × 40—plants treated with BTHCholine 40 mg/L in total of 9 times. The full list of treatments is shown in Table S1 in the ESI.

3.3. Experiment 2022

3.3.1. General Yield

A significant increase in marketable yield compared to plants of SFP was observed for all tested variants of treatment of both tested substances (Table 7). Only in the case of treatment, according to variant BTHCholine 9 × 40, the difference is not statistically proven. As for the non-marketable yield, a significant increase was observed only for variants of BTHWA 6 × 20, BTHCholine 6 × 20 and BTHCholine 6 × 40. When it comes to total yield, only treatment according to variant BTHWA 9 × 40 and BTHCholine 9 × 40 did not result in a significant increase compared to variant SFP. For all tested variants of treatment of both tested substances, the ratio of non-marketable to marketable yield was decreased compared to the SFP variant.

Table 7. Quantitative parameters describing the yield as a result of treatment according to tested variants.

	Marketable Yield [t/ha]	Non-Marketable Yield [t/ha]	Total Yield [t/ha]	Ratio of Non-Marketable to Marketable Yield [%]
SFP	64.96 ± 1.14 a	6.44 ± 0.11 a	71.40 ± 1.25 a	9.92
BTHWA 6 × 20	79.00 ± 2.30 d	7.46 ± 0.51 b	86.46 ± 2.81 c	9.44
BTHWA 9 × 20	73.46 ± 1.39 c	6.79 ± 0.17 ab	80.25 ± 1.56 b	9.24
BTHWA 6 × 40	75.40 ± 0.85 cd	6.78 ± 0.12 ab	82.18 ± 0.97 b	8.99
BTHWA 9 × 40	69.46 ± 1.32 b	6.07 ± 0.31 a	75.53 ± 1.64 a	8.74
SFP	64.96 ± 1.14 a	6.44 ± 0.11 a	71.40 ± 1.25 a	9.92
BTHCholine 6 × 20	75.7 ± 2.75 c	7.22 ± 0.39 b	82.92 ± 3.14 c	9.54
BTHCholine 9 × 20	70.96 ± 2.08 bc	7.66 ± 0.15 ab	77.62 ± 2.23 bc	9.39
BTHCholine 6 × 40	75.01 ± 1.80 c	7.2 ± 0.31 b	82.21 ± 2.11c	9.59
BTHCholine 9 × 40	68.66 ± 1.92 ab	6.3 ± 0.16 a	74.96 ± 2.08 ab	9.18

Symbols for the table: for each active substance separately, within the columns, mean values marked with lowercase letters differ significantly depending on the variant of treatments at p = 0.05 according to Tukey's HSD, while mean values are not marked with a letter if differences are not significant. SFP—plants treated with standard fungicide treatment; BTHWA 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHWA 9 × 20—plants treated with BTHWA 20 mg/L in total of 9 times; BTHWA 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHWA 9 × 40—plants treated with BTHWA 40 mg/L in total of 9 times; BTHCholine 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHCholine 9 × 20—plants treated with BTHCholine 20 mg/L in total of 9 times; BTHCholine 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHCholine 9 × 40—plants treated with BTHCholine 40 mg/L in total of 9 times. The full list of treatments is shown in Table S1 in the ESI.

3.3.2. Tomato Yield Structure

The distribution of the number of fruits in each of the diameter classes was significantly affected by tested variants of treatment (Table 8). As in the case of an experiment performed in 2021, these differences are mainly manifested in the number of fruits with diameters of 3.5–4 cm and 4.0–4.5 cm. For all tested variants consisting of treatments either with BTHWA or BTHCholine, the highest number of fruits having a diameter of 3.5–4 cm was observed for fruits of SFP. As for the fruits of a diameter of 4.0–4.5 cm, the lowest amount was observed for fruits of SFP, but the difference compared to all tested variants of treatment is not significant for a variant of BTHWA 9 × 20, BTHCholine 6 × 40 and BTHCholine 9 × 20. Regarding fruit with the highest diameter, a significant increase compared to fruits of SFP was observed only for BTHWA 6 × 20, BTHCholine 6 × 40 and BTHCholine 6 × 40.

Table 8. The number of fruits of different diameters as a result of treatment according to tested variants.

	The Number of Fruits [pcs/plot]			
	Diameter 3.5–4 cm	**Diameter 4.0–4.5 cm**	**Diameter >4.5 cm**	**Sum of All Fruits**
SFP	223 ± 8.98 d	265 ± 12.62 a	373 ± 6.27 a	861 ± 9.48 A
BTHWA 6 × 20	151 ± 15.19 a	308 ± 9.38 c	400 ± 13.88 b	859 ± 27.24 A
BTHWA 9 × 20	176 ± 7.49 bc	281 ± 11.1 ab	385 ± 14.32 ab	842 ± 29.95 A
BTHWA 6 × 40	171 ± 13.51 ab	289 ± 7.57 bc	394 ± 11.11 ab	854 ± 15.42 A
BTHWA 9 × 40	198 ± 9.76 bc	280 ± 7.83 bc	381 ± 5.29 ab	859 ± 8.6 A
SFP	223 ± 8.98 d	265 ± 12.62 a	373 ± 6.27 a	861 ± 9.48 A
BTHCholine 6 × 20	146 ± 11.74 a	321 ± 8.2 c	384 ± 11.43 b	851 ± 31.27 A
BTHCholine 9 × 20	184 ± 6.83 b	295 ± 8.86 b	361 ± 12.25 a	840 ± 27.38 A
BTHCholine 6 × 40	154 ± 8.64 a	267 ± 10.86 a	409 ± 8.76 c	830 ± 27.3 A
BTHCholine 9 × 40	193 ± 10.1 b	276 ± 9.97 ab	376 ± 11.69 ab	845 ± 34.40 A

Symbols for the table: within the columns, mean values marked with lowercase letters differ significantly depending on the variant of treatments at $p = 0.05$ according to Tukey's HSD, while mean values are not marked with a letter if differences are not significant. SFP—plants treated with standard fungicide treatment; BTHWA 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHWA 9 × 20—plants treated with BTHWA 20 mg/L in total of 9 times; BTHWA 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHWA 9 × 40—plants treated with BTHWA 40 mg/L in total of 9 times; BTHCholine 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHCholine 9 × 20—plants treated with BTHCholine 20 mg/L in total of 9 times; BTHCholine 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHCholine 9 × 40—plants treated with BTHCholine 40 mg/L in total of 9 times. The full list of treatments is shown in Table S1 in the ESI.

Of note, despite these differences in terms of the distribution of the number of fruits in each of the diameter classes, no variability was noted in terms of the total number of fruits from the plots.

The total mass of the fruits was also affected by the application of tested substances (Table 9). As for the results of treatment with BTHWA, only fruits of diameter 3.5–4 cm treated with either BTHWA 6 × 20 or BTHWA 9 × 20 had a higher total mass of fruits per plot, compared to this of the SFP variant. As for the fruits of diameter 4–4.5 cm, all of the tested variants had a higher total mass of fruits per plot compared to this SFP variant. The same applies to the fruits of the highest diameter, with the difference that fruits treated according to variant BTHWA 9 × 40 had the same total mass compared to this of the SFP variant. As for the results of treatment with BTHCholine, the fruits of diameter 3.5–4 cm treated with variants of BTHCholine 6 × 20, BTHCholine 9 × 20 and BTHCholine 6 × 40 had a significantly higher total mass of fruits compared to fruits of SFP variant. As for the fruits of diameter 4–4.5 cm, all of the tested variants, except form variant BTHCholine 6 × 40, had a higher total mass of fruits per plot compared to the SFP variant. As regards fruits with the highest diameter, treatment according to all tested variants resulted in a significant increase in the total mass of fruit compared to SFP.

Of note, significant changes were observed for the sum of the total mass of fruits for all three diameter classes. For the BTHWA, a significant increase compared to SFP was observed for all tested variants of treatment, with BTHWA 6 × 20 having the highest sum of total mass. The same applies for variants of treatment with BTHCholine, except from the variant BTHCholine 9 × 40.

Table 9. Total mass of fruits of different diameters as a result of treatment according to tested variants.

	Total Mass of Fruits [kg/plot]			
	Diameter 3.5–4 cm	**Diameter 4.0–4.5 cm**	**Diameter > 4.5 cm**	**Sum**
SFP	7 ± 0.26 c	15 ± 0.44 a	34.18 ± 1.49 a	56.18 ± 1.13 a
BTHWA 6 × 20	5.28 ± 0.54 a	18.73 ± 0.41 d	44.30 ± 2.06 c	68.31 ± 2.29 d
BTHWA 9 × 20	6.2 ± 0.18 b	17.31 ± 0.33 bc	40.02 ± 0.97 b	63.53 ± 1.38 c
BTHWA 6 × 40	6.36 ± 0.23 bc	18.1 ± 0.32 cd	40.75 ± 0.64 b	65.21 ± 0.84 cd
BTHWA 9 × 40	6.70 ± 0.46 bc	16.89 ± 0.48 b	36.46 ± 1.1 a	60.05 ± 1.32 b
SFP	7 ± 0.26 c	15 ± 0.44 a	34.18 ± 1.49 a	56.18 ± 1.13 a
BTHCholine 6 × 20	4.785 ± 0.27 a	19.25 ± 0.58 d	41.42 ± 1.97 c	65.45 ± 2.7 c
BTHCholine 9 × 20	6.18 ± 0.15 b	17.8 ± 0.32 c	37.38 ± 1.64 b	61.36 ± 2.02 bc
BTHCholine 6 × 40	5.19 ± 0.34 a	16.00 ± 0.59 ab	43.67 ± 0.88 c	64.86 ± 1.8 c
BTHCholine 9 × 40	6.54 ± 0.38 bc	16.56 ± 0.46 b	36.27 ± 0.99 b	59.37 ± 1.76 ab

Symbols for the table: within the columns, mean values marked with lowercase letters differ significantly depending on the variant of treatments at $p = 0.05$ according to Tukey's HSD, while mean values are not marked with a letter if differences are not significant. SFP—plants treated with standard fungicide treatment; BTHWA 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHWA 9 × 20—plants treated with BTHWA 20 mg/L in total of 9 times; BTHWA 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHWA 9 × 40—plants treated with BTHWA 40 mg/L in total of 9 times; BTHCholine 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHCholine 9 × 20—plants treated with BTHCholine 20 mg/L in total of 9 times; BTHCholine 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHCholine 9 × 40—plants treated with BTHCholine 40 mg/L in total of 9 times. The full list of treatments is shown in Table S1 in the ESI.

An increase in the average mass of fruit resulting from the application of tested variants of treatment was observed for both tested substances (Table 10). A significant increase in the average mass of fruit compared to fruits of SFP was observed for tomato fruits of every diameter class treated with all variants of treatment with BTHWA, except from the variant BTHWA 9 × 40. In general, results for variants of treatment with BTHCholine follow the same trend, although not all differences are statistically proven. For fruits with the highest diameter, the use of all treatment variants resulted in a significant increase in the average fruit weight compared to SFP.

Table 10. Average mass of fruit of different diameters as a result of treatment according to tested variants.

	Average Mass of Fruit [g]			
	Diameter 3.5–4 cm	**Diameter 4.0–4.5 cm**	**Diameter >4.5 cm**	**Weighted Average of Fruit [g]**
SFP	31.40 ± 1.0 a	56.65 ± 1.09 a	91.60 ± 2.46 a	65.26 ± 2.0 a
BTHWA 6 × 20	35.00 ± 0.38 bc	60.84 ± 0.34 b	110.73 ± 2.32 c	79.55 ± 1.87 d
BTHWA 9 × 20	35.24 ± 0.55 bc	61.64 ± 0.66 b	103.99 ± 1.39 b	75.48 ± 1.11 c
BTHWA 6 × 40	37.27 ± 1.66 c	62.64 ± 0.31 b	103.45 ± 1.44 b	76.36 ± 0.52 c
BTHWA 9 × 40	33.86 ± 0.95 b	60.34 ± 0.87 b	95.7 ± 2.22 a	69.92 ± 1.24 b
SFP	31.40 ± 1.0 a	56.65 ± 1.09 a	91.60 ± 2.46 a	65.26 ± 2.0 a
BTHCholine 6 × 20	32.83 ± 1.16 ab	59.98 ± 0.35 b	108.33 ± 5.1 b	77.15 ± 2.86 c
BTHCholine 9 × 20	33.62 ± 0.54 b	60.37 ± 0.37 b	103.51 ± 1.25 b	73.05 ± 0.2 b
BTHCholine 6 × 40	33.71 ± 0.9 b	59.95 ± 0.15 b	106.79 ± 0.85 b	78.17 ± 0.47 c
BTHCholine 9 × 40	33.9 ± 0.49 b	60.01 ± 0.48 b	96.47 ± 1.31 a	70.28 ± 0.51 b

Symbols for the table: within the columns, mean values marked with lowercase letters differ significantly depending on the variant of treatments at $p = 0.05$ according to Tukey's HSD, while mean values are not marked with a letter if differences are not significant. SFP—plants treated with standard fungicide treatment; BTHWA 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHWA 9 × 20—plants treated with BTHWA 20 mg/L in total of 9 times; BTHWA 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHWA 9 × 40—plants treated with BTHWA 40 mg/L in total of 9 times; BTHCholine 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHCholine 9 × 20—plants treated with BTHCholine 20 mg/L in total of 9 times; BTHCholine 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHCholine 9 × 40—plants treated with BTHCholine 40 mg/L in total of 9 times. The full list of treatments is shown in Table S1 in the ESI.

Of note, weighted average values for the average fruit weight indicate a significant increase compared to the SFP variant for all tested variants for treatments for both substances. Among treatments with BTHWA, the application of BTHWA 6 × 20 resulted in the highest value of this parameter. Among treatments with BTHCholine, the highest value of this parameter was observed for variants BTHCholine 6 × 20 and BTHCholine 6 × 40.

3.3.3. Bioactive Substances

Results describing the content of lycopene and total soluble solids in fruit from experiments conducted in 2022 follow a similar trend as those obtained in 2021. As for the content of lycopene in tomato fruits, a significant increase compared to fruits of SFP is observed for all variants of treatment with BTHWA. Fruits treated according to variants BTHCholine 6 × 20 and BTHCholine 9 × 20 had a significantly higher content of lycopene compared to fruits of SFP.

Treatment of plants according to variants BTH. An increase in TSS content was also observed for the remaining variants of treatment, but this difference is not significant. (See Table 11).

Table 11. The content of lycopene and total soluble sugar (TSS) in tomato fruits was treated according to tested variants of treatment.

	Lycopene [mg/100 g/DW]	**TSS [°Brix]**
SFP	3.82 ± 0.1 a	6.82 ± 0.06 a
BTHWA 6 × 20	4.48 ± 0.05 bc	7.47 ± 0.07 b
BTHWA 9 × 20	4.44 ± 0.07 bc	7.35 ± 0.08 b
BTHWA 6 × 40	4.38 ± 0.05 bc	7.19 ± 0.07 ab
BTHWA 9 × 40	4.16 ± 0.06 b	7.04 ± 0.23 ab
SFP	3.85 ± 0.1 a	6.82 ± 0.06 a
BTHCholine 6 × 20	4.40 ± 0.06 b	7.40 ± 0.05 b
BTHCholine 9 × 20	4.2 ± 0.08 b	7.37 ± 0.07 b
BTHCholine 6 × 40	4.07 ± 0.05 ab	7.28 ± 0.09 ab
BTHCholine 9 × 40	4.06 ± 0.04 ab	7.12 ± 0.16 ab

Symbols for the table: for each active substance separately, within the columns, mean values marked with lowercase letters differ significantly depending on the variant of treatments at p = 0.05 according to Tukey's HSD, while mean values are not marked with a letter if differences are not significant. SFP—plants treated with standard fungicide treatment; BTHWA 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHWA 9 × 20—plants treated with BTHWA 20 mg/L in total of 9 times; BTHWA 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHWA 9 × 40—plants treated with BTHWA 40 mg/L in total of 9 times; BTHCholine 6 × 20—plants treated with BTHWA 20 mg/L in total of 6 times; BTHCholine 9 × 20—plants treated with BTHCholine 20 mg/L in total of 9 times; BTHCholine 6 × 40—plants treated with BTHWA 40 mg/L in total of 6 times; BTHCholine 9 × 40—plants treated with BTHCholine 40 mg/L in total of 9 times. The full list of treatments is shown in Table S1 in the ESI.

4. Discussion

The presented results indicate that as a result of application according to tested variants of treatment for both BTHWA and BTHCholine, which are the functional analogues of salicylic acid, the phenomena of growth immunity tradeoff did not occur. The result proved interesting as not only was there no decrease in tomato quantitative and qualitative parameters of yield, but also, for most of the tested variants of treatment, an increase in these parameters was observed. As already mentioned, the original aim of this study was to even provoke a growth-immunity tradeoff to measure to what extent it will affect yield. However, it turned out that even for the concentration twice as high as optimal and for the total of 9 treatments with testes substances, no negative effect on tomato yield was observed. This provides a basis to conclude that the tested substances have biostimulating activity.

The biostimulating effect of one of these compounds was previously reported by us [17]. When BTHWA was applied to tulips in 4 watering treatments, a double effect of this substance was observed, i.e., providing effective protection against *Fusarium oxysporum* f. sp. *tulipae* and a significant increase in the size of the plants. Moreover, the same effect was observed for both test concentrations of BTHWA, i.e., 20 mg/L and 40 mg/L.

It is noteworthy that the BTHWA-treated plants exhibited a significant increase in height compared to both the untreated control that was inoculated and the untreated control that was not inoculated. The same trend was observed for the fresh mass and dry mass of the aboveground part of the plant. This method of application was considered as appropriate as the growth-immunity trade-off was not observed.

Other reports indicate that plant growth is reduced by the exogenous application of salicylic acid or its analogues, i.e., benzothiadiazoles [20,21]. Moreover, it has been reported that the BTH treatment of tomato, cucumber, or beans with the same doses and the same frequency of application has brought about different effects on the vegetative development, size of plants and yield [22]. Plant size or yield of tomato plants was not affected by treatment with different concentrations of BTH, except for the treatment with BTH in a concentration of 1000 mg/L that resulted in a low level of leaf stunting and a slight leaf scorching. As for the bean and cucumber, the application of BTH in the concentration of 100 mg/L resulted in a reduction of plant size, a decrease in plant growth rate, and a reduced number of flowers and fruits. The same response was observed for plants treated with higher concentrations of BTH. The results presented in this study are in line with other results on field experiments on tomatoes. Rożek et al. performed a field study on five different tomato varieties, including the variety Dyno F1 [23]. Obtained results for marketable yield, non-marketable yield and total yield for variants of treatment consisting of standard protection with plant protection products are in a similar range compared to these presented in our study. The same applies to parameters describing number of fruits and their diameter. Increased content of TSS was also reported by Huan et al. [24]. As for the increase in lycopene content, it was reported by Iriti et al. that the content of lycopene was increased as a result of treatment with BTH [25]. Similar results were observed for the treatment of tomato plants with salicylic acid [26,27].

As for the lycopene itself, this is a compound of significant importance not only in terms of assessing the quality of tomato fruit but also in the health-promoting context for humans. Lycopene is a carotenoid that is responsible for the enhancement of protection against cardiovascular, neurodegenerative and inflammatory diseases, as well as cancer and hypertension [28,29]. As for the content of lycopene in tomatoes, it ranges from 0.88 to 7.74 mg/100 g and depends on the species and ripening stage [30,31]. However, each method allowing for an increase in the lycopene content in tomatoes is of key importance and scientific significance. If such methods become common, which is somewhat imposed by the assumptions related to the development of effective agriculture, all consumers will benefit as they will be able to consume larger amounts of this ingredient [32,33].

To address the issue of sustainability presented in this work, firstly, it is to be considered with reference to active substances themselves. Their parent compound is benzo[1.2.3] thiadiazole-7-carboxylic acid, *S*-methyl ester (BTH), an active substance of products Bion 50 WG and Actigard 50 WG by Syngenta company. Due to regulatory requirements, the BTH and its metabolites (such as benzo[1,2,3]thiadiazole-7-carboxylic acid) have been extensively investigated for their safety and use and have passed the registration procedure as plant protection products. Both tested substances, i.e., BTHWA and BTHCholine, undergo degradation to benzo[1,2,3]thiadiazole-7-carboxylic acid. As for the BTHWA substance, its functional group is based on *N*,*O*-dimethylhydroxylamine that is methylated hydroxylamine used to form so-called 'Weinreb amides' for use in the Weinreb ketone synthesis [34]. Information related to the safety issues of this substance is not provided in its MSDS [35]. As BTHWA was one of the most promising active substances developed by us, appropriate tests regarding the safety of its use have been carried out, the results of which will be the subject of a separate scientific publication. Among other tests, an acute oral toxicity study on rats—Acute Toxic Class Method according to the OECD Guideline No. 423/EU Method B.1.TRIS was performed. According to unpublished data, BTHWA can be classified into "category 5 or beyond classification" (range defined as 2000 mg/kg < LD50 < 5000 mg/kg) according to the Globally Harmonized System (GHS). Similar results are expected for BTHCholine as the anion core remains the same as in the case of BTHWA.

The cation of BTHCholine is choline, which is an essential nutrient for humans and many other animals and was formerly classified as a B vitamin [36]. In the MSDS for choline chloride, the LD_{50} as high as 3900 mg/kg is indicated [37]. What is more, BTHCholine, being an ionic derivative, will most likely be safer compared to neutral derivatives of BTH. In our previous work, we reported the investigation of a proactive hazard assessment of eleven BTH derivatives that was performed in parallel with technological development to screen for possible environmental hazards [38]. Ready biodegradability, cytotoxicity and aquatic toxicity were employed as a starting point. As a result, BTHCholine showed both lower (eco)toxicity and higher biodegradability than the parent compound. BTHWA compound was not subject to this study. Another positive effect of substance derivatisation to ionic form was observed in our work, in which we examined the level of substance absorption by plants. The results indicated that the ionic derivative was absorbed to a lesser extent in the plant compared to the parent compound in non-ionic form.

As for the issue of sustainability related to the general idea of using tested active substances, it can be considered as the use of BTHWA and BTHCholine as both SAR inducer and biostimulant. Firstly, the use of SAR inducers as a plant protection strategy has a number of advantages over the application of PPPs. The mechanism of SAR induction is common to all plants. In our previous studies on BTHWA, we proved the efficiency of SAR induction on various plants, such as strawberries and zucchini. Results of experiments that were conducted on other plants, such as wheat, rapeseed, sugar beet and cucumber, are soon to be published. Moreover, the use of SAR inducers overcomes the problem of pathogen resistance, as pathogens are not a direct target of SAR inducers. What is more, it endows long-term resistance against a broad spectrum of pathogens, which continues even after the application of SAR inducers is terminated. It usually lasts for weeks after the final application as a result of the stimulation action of the inducer on the plant's natural defence mechanisms. Compared to PPPs that are commonly used in doses of at least 250 g per hectare of crop, SAR inducers are used in much smaller doses. As for the concentration of active substances used in this work, a concentration of 20 mg/L in a working solution of 500 L per hectare corresponds to 10 g of the substance per hectare, while a concentration of 40 mg/L corresponds to 20 g per hectare of crop. An additional issue related to sustainability is associated with the EU legislation on ecological development and natural environment protection. EU policies include curbing the use of pesticides to reduce their impact on human health and the environment, as well as incentivising alternative approaches and techniques. The desire to reach these goals is expressed in regulations 1107/2009 and 128/2009, which set out legal requirements defining the active substances allowed for use in agricultural practice. As a result, the number of pesticides available for agricultural production has declined. The first to be eliminated were the substances most toxic to the natural environment, although they are often the most effective against pathogens. Their elimination has stimulated the search for new and effective methods of plant protection, employing the use of novel active ingredients that satisfy legal requirements, environmental issues, and societal expectations. What is more, the need for sustainable development in the context of pesticide use is also demonstrated in "Farm to Fork Strategy", which indicates a goal of 50% reduction in pesticide use by 2030. In our previous work, we demonstrated such a possibility with the use of another ionic derivative named choline 3,5-dichlorosalicylate (3,5 diClSal) in the cultivation of sugar beet [39]. A similar level of protection against C. beticola and the amount of technological sugar yield was obtained as a result of treatment with a combined variant consisting of SAR inducer and fungicide (in a reduced number of treatments) as well as a variant consisted of standard fungicide treatment (SFP). The SFP variant consisted of two applications of fungicides, while combined variants of SAR inducer and fungicide consisted of either 4 or 3 applications of 3,5 diClSal and one application of a fungicide. Concerning the two mentioned variants involving the combined use of fungicide and SAR inducer, the variant assuming four treatments proved to be better than the variant assuming three treatments. Hopefully, it will be possible to demonstrate the possibility of achieving such a goal in future experiments on BTHWA or BTHCholine substances as part of subsequent experiments carried out in the cultivation of different crops. However, it should be kept in mind that PPPs have different active substances and have different modes of action. Therefore, to develop such a program for the protection of a given crop, it will be necessary to engage an agrochemical company that will incorporate a SAR inducer into its protection programs. At this stage of SAR inducer development, it is of key necessity to perform research aimed at determining the optimal possible technology of the use and to investigate other related issues, especially those considering the economic aspects related primarily to the number of treatments necessary.

Secondly, tested active substances can also be considered as having the activity of biostimulants. As follows from the definition, a plant biostimulant is any substance or microorganism applied to plants with the aim of enhancing nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content [40]. The presented results provide the basis for conducting more detailed research on tested substances in terms of indicated activities. Of particular importance is the activity related to increasing the efficiency of fertilisation, as it can contribute to meeting the assumption of reducing the use of fertiliser by at least 20%, which is also indicated in "The Farm to Fork Strategy".

Each of the mentioned activities can be investigated separately, but the ultimate confirmation of the activity of a given substance in the tested application technology will have to be made in an experiment conducted in field conditions, in which aspects related to both the effectiveness of SAR induction and the plant biostimulation are simultaneously examined. Until now, we have demonstrated that the application of BTHWA on tulips resulted in both efficient SAR induction and stimulation of plant growth [17].

The stage of research presented in this article refers to searching for the optimal application technology of tested active substances that are related to the total number of treatments and concentration of active substances corresponding to dose per hectare. The long-term goal of our research activities is to introduce to the market an active substance with SAR induction and biostimulation properties. During the research, some of the gaps in the literature were covered. Firstly, the most widely studied substances are still BTH and Salicylic acid. From a chemical perspective, these substances are functional analogues with a corresponding spherical-electron arrangement. Thus, it is possible to introduce such a target modification into the chemical structure of the compound that will not change this arrangement and will improve the physicochemical properties of the given compound. As a result, a completely new chemical compound is obtained. As for our previous research in this area, we conducted research on cationic or anionic derivatives, proving that target modification can result in the improvement of the physicochemical properties of a substance while maintaining or even improving its biological activity [14,15]. Another research gap that is covered by our research is related to the fact that agricultural experiments on SAR induction are mostly performed in controlled conditions, i.e., in a greenhouse, rather than in field conditions. Research on the activity of biostimulants is conducted in field conditions, but according to the definition related to biostimulants, the activity of such substances related to ensuring effective protection against diseases is not always the subject of such research.

We hope that our results will be of interest to the scientific community, whose involvement is necessary to conduct detailed research related to the effectiveness of the tested substances on various crops grown in various growing conditions, such as geographical location or soil conditions. The scope of these studies will be additionally extended by the need to simultaneously analyse the activity associated with the induction of plant resistance against various diseases and biostimulating activity understood as stimulation of quantitative and qualitative parameters of crops, mitigating the negative effects of abiotic stresses and increasing the efficiency of nutrient uptake. Only the use of a given substance in its optimal way, as well as thorough research on the plant response, will allow the full potential of these molecules to be discovered.

5. Conclusions

This study presents results for the stage of research that is related to the investigation of the most optimal technology of application of two novel active substances named BTHWA and BTHCholine. These substances were originally treated only as SAR inducer; however, according to collected evidence, the application of these substances is also manifested in plant biostimulation. Variant of treatment tested assumed a total of 6 or 9 times in a program combined with a standard protection program. Moreover, two different doses of both substances were tested, one of them being the standard dose established in previous studies, while the second dose was twice as high as the one optimally used. This was intended to cause a reduction in plant yield as a result of plant metabolism disruption. However, the results proved very interesting as they stand in contrast to other results obtained in our previous research and by other authors investigating plant resistance induction. Even the application of tested substances in concentration twice as high as the one optimally used and in a total of nine treatments did not result in the occurrence of a growth-immunity tradeoff. For the remaining variants of treatment, a positive influence on the qualitative and qualitative parameters of yield was observed.

Results of this study indicate the potential of BTHWA and BTHCholine as active substances having the activities of both SAR inducers and biostimulants. This provides the basis for further investigation on the development of the technology of their use that can be incorporated into plant protection programs with the intention of both reduction of plant protection products used as well as stimulation of qualitative and quantitative parameters of yield. Both these potential results are in line with EU policies related to sustainable development, such as the "Farm to Fork Strategy".

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su16125191/s1, Table S1: Schedule of treatments for the experiment in 2021 and 2022.

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