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Preliminary Tests of Tomato Plant Protection Method with Ozone Gas Fumigation Supported with Hydrogen Peroxide Solution and Its Effect on Some Fruit Parameters

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Abstract: The aim of this research was to determine the impact of hydrogen peroxide spraying and ozone gas fumigation during the growing season of tomato plants grown under cover on the mechanical and chemical parameters of fruit harvested from these plants. Tomato plants were grown under cover in accordance with the principles of good agricultural practice in the soil and climatic conditions of southeastern Poland. During the growing season, tomato fruits were collected for testing in order to determine the impact of the applied variable factors on the modification of selected metabolic pathways of bioactive compounds. As part of the tests on the chemical properties of the fruits, the content of ascorbic acid, the total content of polyphenols, and the antioxidant potential were determined. Additionally, the influence of the tested variable factors on the mechanical properties of tomato fruits was determined. In the case of the total polyphenol content, the most beneficial effects were observed for fruits collected from plants treated with ozonation at a dose of 2 ppm for 3 min and spraying the plants with 1% hydrogen peroxide. The highest antioxidant potential was recorded for fruits of the variants ozonated with doses of 2 ppm for 1 min, 2 ppm for 1.5 min, and 2 ppm for 3 min compared to the remaining variants and controls. In turn, the vitamin C content increased significantly in the tested fruits after the ozonation of plants with a dose of 2 ppm for 1 min and ozonation with a dose of 2 ppm for 3 min combined with spraying plants with 3% hydrogen peroxide. In the case of the mechanical properties of tomato fruits, only the ozonation dose of 2 ppm for 3 min significantly improved them.

Keywords: tomato fruit; ozone gas; hydrogen peroxide; chemical properties; residue-free methods; sustainable agriculture; sustainable food production

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1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is cultivated in an area spanning 5.1 million hectares across the world. Due to their numerous properties and wide culinary use, tomato fruits are very popular among consumers [1,2]. The main producer of tomatoes is China, with a production of 64.9 million tons, followed by India with 20.6 million tons, Turkey with 13.2 million tons, and the USA with 12.2 million tons [1]. Tomatoes are consumed largely as processed products, e.g., ketchup and purees. Recently, there has been increased interest in the use of tomato seeds [3,4] to produce oils with a high content of unsaturated fatty acids [5]. Tomato is considered an excellent source of bioactive compounds, especially carotenoids, such as lycopene and β-carotene, ascorbic acid, tocopherols, and flavonoids, which have high antioxidant capabilities [6]. The high content of antioxidants present in tomatoes and tomato products is associated with a reduced risk of certain types of cancer and cardiovascular diseases [7–9].

Attention to the quality of the products of plant origin and the production environment in agriculture and horticulture has led to the withdrawal of many plant protection products from crops. Currently, there is increasing interest in biocides that leave no residues in the produced plant raw materials and are non-toxic to the natural environment [10]. When growing plants in both field and sheltered conditions, they are exposed to various biotic and abiotic stress factors. It is worth noting that plants have developed mechanisms that allow them to adapt to environmental stressors. Additionally, plants have developed stress tolerance mechanisms [11,12]. Currently, unconventional methods of plant protection that do not pollute the natural environment and produced food are currently in demand. Some hopes are associated with hydrogen peroxide [10] or ozone gas [13], which are perceived as stress factors, but an appropriately selected dose of each stress factor can bring benefits without causing losses during plant cultivation.

Due to its properties, hydrogen peroxide has a number of applications in the medicine, cosmetics, food, chemical, paper, and textile industries. Additionally, it is commonly used to disinfect water [14]. It is considered a "green industrial oxidant" because it leaves no harmful residues and quickly decomposes into water and oxygen molecules [15]. The use of hydrogen peroxide in sustainable agriculture is due to the fact that it fights harmful microorganisms, modifies the internal resistance of plants to biotic and abiotic stresses, and affects plant growth. In plant production, H_2O_2 is used as a compound to combat viral, bacterial, and fungal pathogens and plant pests. Additionally, it is an excellent agent for disinfecting storage rooms and protecting plant products intended for storage [16]. The action of hydrogen peroxide is associated with the activation of immune functions in plants. Its action results in the strengthening of cell walls, increased growth and development of plants, and production of growth regulators and phytoalexins [17,18]. In medicine, hydrogen peroxide solutions up to 3% are used, while in cosmetology 6% solutions are used, but only externally. In the case of plants, hydrogen peroxide concentrations of up to 18% have been used, but good effects are already observed at lower concentrations [19].

As a result of normal metabolic reactions, reactive oxygen species (ROS) are produced in plants [20,21], which occur in free radical and non-radical forms. Non-radical forms, including hydrogen peroxide, have a half-life in various types of systems, including biological ones. Its action may have a longer effect on the plant. Radical forms, including the hydroxyl radical, are extremely reactive, and their presence usually leads to serious damage to tissues or cellular systems. The overproduction and accumulation of ROS is the result of primarily biotic and abiotic stresses [22,23]. The result of high levels of ROS is damage to plant cells, which may ultimately lead to the destruction of the entire plant [24,25]. H_2O_2 can cause damage by oxidizing various macromolecules and enzymes of the Calvin–Benson cycle. Biotic and abiotic stress contribute to the accumulation of reactive oxygen species and may be the main cause of loss of crop productivity [26,27].

Ozone was approved by the US Food and Drug Administration in 2001 as a disinfectant, which resulted in great interest in this gas from various industrial sectors and scientists [28]. Ozone gas has antibacterial properties and is non-toxic, which is why it is widely used in the food industry [29]. It is also possible to use ozone dissolved in water [30]. It is important to determine the appropriate dose of ozone that will have a beneficial effect on selected fruits or vegetables. Ozone gas has been tested in many studies and found to have a positive effect on the storage life and quality of stored raw materials [31,32]. Ozone gas eliminates pathogen spores, thereby reducing the microbial population [28,33]. Additionally, it is used to reduce the burden of mycotoxins and pesticide residues [34,35]. The exposure of plants and harvested fruits and vegetables to O_3 during vegetation causes metabolic changes. Therefore, the biosynthesis of secondary metabolites takes place, such as polyphenols in the plants themselves and products of plant origin. The amount and type of secondary metabolites produced are closely related to various factors, e.g., the dose of ozone or the variety of plant treated with this gas [36,37]. Currently, many studies are related to the possibility of using ozone as an abiotic stressor that can be used to influence the content of bioactive compounds in plants and plant products [28,38].

Currently, there are many scientific studies available that report the effects of ozone on plants. Ozone affects the growth of plants and their metabolism. The effect of ozone gas on plants depends on the concentration of this gas, the duration of exposure, and the type of plant [39,40]. Generally, plant responses to ozone exposure are determined mainly by the level of oxidative cell damage [41]. As a result of toxic doses of ozone, oxidative stress increases and leads to the reduced transcription of genes encoding proteins involved in photosynthesis and ultimately promotes leaf degradation [42,43]. Scientific reports indicate that high exposure to ozone may have a negative impact on the chlorophyll content in the green parts of plants [44,45]. In the case of plants grown for economic purposes, determining the impact of ozone gas is primarily based on yield losses [46].

In the case of ozone gas, a safe concentration cannot be clearly determined. The ozone dose, i.e., exposure time and concentration, is important. The negative effects of ozone on plants can already be observed at a few ppb during long exposure to this gas [47]. In addition, plants can withstand several hundred ppm without harm with short exposure [48].

The aim of this study was to assess the impact of foliar spraying with various concentrations of H_2O_2 and fumigation with ozone gas on the selected fruit parameters of tomato plants and to determine safe doses of variable factors that can be used in tomato cultivation under cover.

2. Materials and Methods

2.1. Plant Material and Tomato Growing Conditions

2.1.1. Plant Material in a Field Experiment under Cover

The field experiment was conducted for two years on tomatoes of the Remiz F1 variety (*Solanum lycopersicum*). The variety grown as part of the experiment in question is dedicated to cultivation in greenhouses and foil tunnels.

A field experiment under cover was assumed for four variants:

- Control sample—no variable factors were used (control);
- Variant of plants sprayed with hydrogen peroxide—only hydrogen peroxide sprays were used (W1—1% H_2O_2; W2—3% H_2O_2);
- Variant of ozonated plants—fumigation with ozone gas was carried out (W3—2 ppm 1 min; W4—2 ppm 1.5 min; W5—2 ppm 3 min);
- Variant of plants ozonated and sprayed with hydrogen peroxide—fumigation with ozone gas and hydrogen peroxide spraying were carried out (W6—2 ppm 1 min and 1% H_2O_2; W7—2 ppm 1.5 min and 1% H_2O_2; W8—2 ppm 3 min and 1% H_2O_2; W9—2 ppm 1 min and 3% H_2O_2; W10—2 ppm 1.5 min and 3% H_2O_2; W11—2 ppm 3 min and 3% H_2O_2). See Supplementary Materials: S4.

2.1.2. Tomato Growing Conditions

For each year of the experiment, the fore crop for tomatoes was garlic. Fertilization doses for tomatoes were developed based on soil analysis. In autumn, phosphorus–potassium fertilization was applied, and tillage treatments were carried out. Pre-sowing cultivation was carried out in spring. Then, the foil tunnels were installed. The gardening foil used in the experiment was made of polypropylene. Tomato seedlings were planted under cover at the beginning of May of each year of the study. The plants were planted in rows at a distance of 50 cm, leaving a 100 cm wide passage between the rows (Figure 1). Fertilization was applied based on soil tests (see Supplementary Materials: S5). During the period of intensive flowering and fruiting, sprays containing microelements were used. Tomato seedlings were grown "for one shoot", i.e., the growing side shoots were regularly removed to obtain high fruit yields. Additionally, excess leaves were removed for better ventilation and improved light conditions in the lower parts of the plants. In mid-August, the growth tips of the main shoots, above the last developed inflorescence, were removed. No additional lighting or heating was used when growing tomatoes.

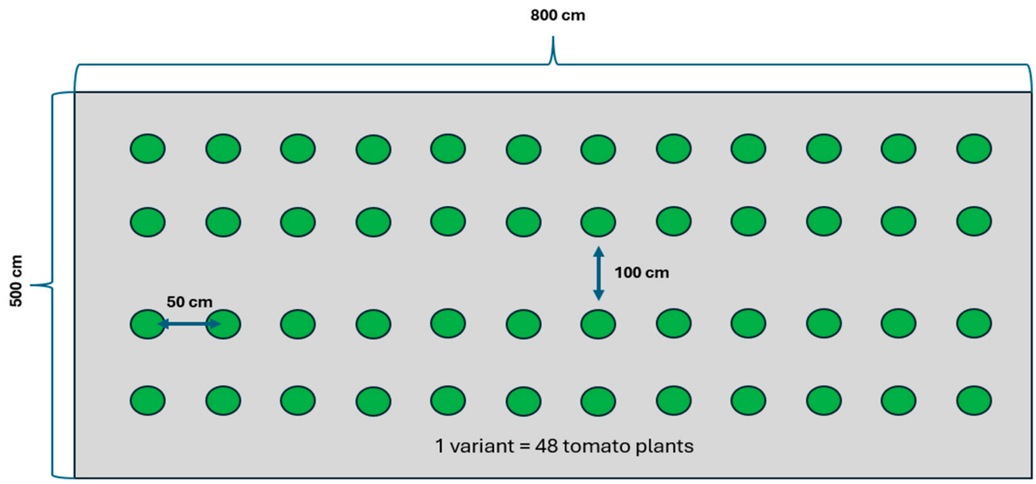

Figure 1. Experiment scheme for each variant (green circles indicate plants).

2.2. Experimental Treatments Used during Plant Vegetation

2.2.1. Ozonation of Tomatoes Plant

Before establishing the field experiment, preliminary tests were carried out to determine the tolerance threshold of tomato plants of the Remiz variety to the gaseous ozone. For this purpose, seedlings of the tested variety were produced and exposed to ozone gas at a concentration of 2, 5, 10, 20, 40, and 100 ppm for 1, 3, 5, and 10 min. Based on the results of the preliminary tests, possible doses of ozone were selected. As part of the experiment, ozone gas was used at a concentration of 2 ppm for 1, 1.5, and 3 min because no phytotoxic effects were observed on the tested plants under these conditions. The foil tunnels in which the experiment was conducted were equipped with a system of channels that enabled the supply of ozone to each plant. The foil tunnel was closed during fumigation, and ozone gas was supplied via a hose from the Korona L5 ozone generator (Korona Science and Implementation Laboratory, Piotrków Trybunalski). In the process of the fumigation of tomato seedlings, the concentration of ozone gas was measured with a 106 M 2B Technologies ozone detector (2B Technologies, Broomfield, CO, USA), the measurement range of which was 0–1000 ppm. The experiment under cover was carried out in three independent repetitions. Ozonation was carried out once a week. Then, after the fumigation process, the foil tunnels were opened and ventilated using mechanical ventilation, completely removing ozone within 20 s.

2.2.2. Spraying with Hydrogen Peroxide (H_2O_2)

Before establishing the field experiment, preliminary tests were carried out to determine the tolerance threshold of tomato plants of the Remiz variety to various concentrations of hydrogen peroxide. For this purpose, a hydrogen peroxide solution with concentrations of 1, 3, 5, and 10% was applied to the produced seedlings. Based on the results of the preliminary tests, possible doses of hydrogen peroxide were selected (solution with concentrations of 1% and 3%), which did not have a phototoxic effect on the tested plants. For the ozonated and sprayed-with-hydrogen-peroxide tomato plants variant and the variant for which only hydrogen peroxide spraying was applied during the growing season, hydrogen peroxide at concentrations of 1% and 3% was used once a week. Moreover, each time after the ozonation process for the "ozonation + hydrogen peroxide" variant, tomato plants were sprayed with a 1% and 3% hydrogen peroxide solution. A hand-held backpack sprayer was used for the spraying treatments. Depending on the growing season, from 10 to 200 mL of spray liquid was used per tomato plant. The hydrogen peroxide solution was prepared immediately before spraying. The spray solution was prepared from 40% perhydrol (Chempur, Gdańsk, Poland).

2.3. Measurement of Mechanical Properties

Tests on the tissue resistance of ripe red tomato fruits to mechanical damage in the process of uniaxial compression, taken for testing in August, were carried out on fruit samples of similar sizes. The measurements were carried out on the control raw material and the raw material tested with variable factors. Measurements were carried out in 36 repetitions for a given combination of the variable factor used. The process of the uniaxial compression of tomato fruits was carried out on a Zwick/Roell Z010 testing machine for the established operating parameters: initial sample stress force F = 2 N; module speed during compression measurement $V = 0.5\ mm \cdot s^{-1}$. Compression tests of the samples were carried out at the height of the tomato fruit, where its diameter is the largest.

2.4. Antioxidant Potential, Contents of Polyphenols, and Vitamin C

The content of polyphenols in freshly harvested tomato fruits was measured according to the methodology described by Matłok et al. [49] using the Folin–Ciocalteu method. The total ascorbic acid content and DPPH antioxidant activity in freshly harvested tomato fruits were determined according to the methodology described by Panich et al. [50]. The analysis was performed in triplicate. In order to determine chemical properties, 500 g of fruit from each experimental variant was used for the analyses. See Supplementary Materials: S1–S3.

2.5. Statistical Analysis

In order to check the significance of the effect of gaseous ozone and yeast spray on the physiological parameters and disease infestation, a two-factor analysis of variance was used for each measurement at the significance level of $\alpha = 0.05$. These analyzes were performed using STATISTICA 13.1 (TIBCO Software Inc., Hillview Avenue, Palo Alto, CA, USA).

3. Results and Discussion

The impact of the methods used to support the protection of tomato plants using gaseous ozone (O_3) and hydrogen peroxide (H_2O_2) on the total content of polyphenols in the collected fruits over the years of this research is shown in Figure 1. It was found that regardless of the conditions of the ozonation process (time of exposure of plants to O_3) (variant W5–W3) and the concentration of H_2O_2 solution (variant W1–W2), no significant differences were recorded in the total content of polyphenols in tomato fruits harvested at harvest maturity (Figure 2). In the case of using a combined method consisting of the initial fumigation of tomato plants with ozone gas and then the foliar application of solutions with different H_2O_2 concentrations, an influence of this method on the total content of polyphenols in the fruit was found. However, this effect varied depending on the H_2O_2 concentration used. In the case of the application of a solution with a lower concentration (1% H_2O_2), a significant increase in the content of polyphenols in fruits was noted, regardless of the time of exposure of the plants to O_3 (variant W6–W8).

The mechanism of the modification of the intensity of the biosynthesis of the phenolic compounds as a result of the combined method of protecting tomato plants using O_3 and H_2O_2 is probably related to the increase in the supply of H_2O_2 to plant tissues as a result of the preceding action of ozone gas. It has been proven that in the event of photochemical smog, ozone gas affects the degree of the opening of the stomata, which significantly facilitates the penetration of vapors containing H_2O_2 or atomic oxygen resulting from its decomposition. These factors have proven activity of inducing oxidative stress, which, under controlled conditions, may lead to the modification of the biosynthesis of small molecular antioxidants, including phenolic compounds [51]. The observed effect of the increased biosynthesis of these compounds in tomato fruits harvested from plants exposed to lower concentrations of H_2O_2 indicates that the effect of the disturbed homeostasis caused the plant defense effect, the consequence of which was an increase in the content of phenolic compounds in the collected raw material. It cannot be ruled out that in the case of higher concentrations of H_2O_2, there was no intensification of the biosynthesis of this group of compounds, but they were decomposed under the influence of higher concentrations of H_2O_2, or its decomposition products found in plant tissues.

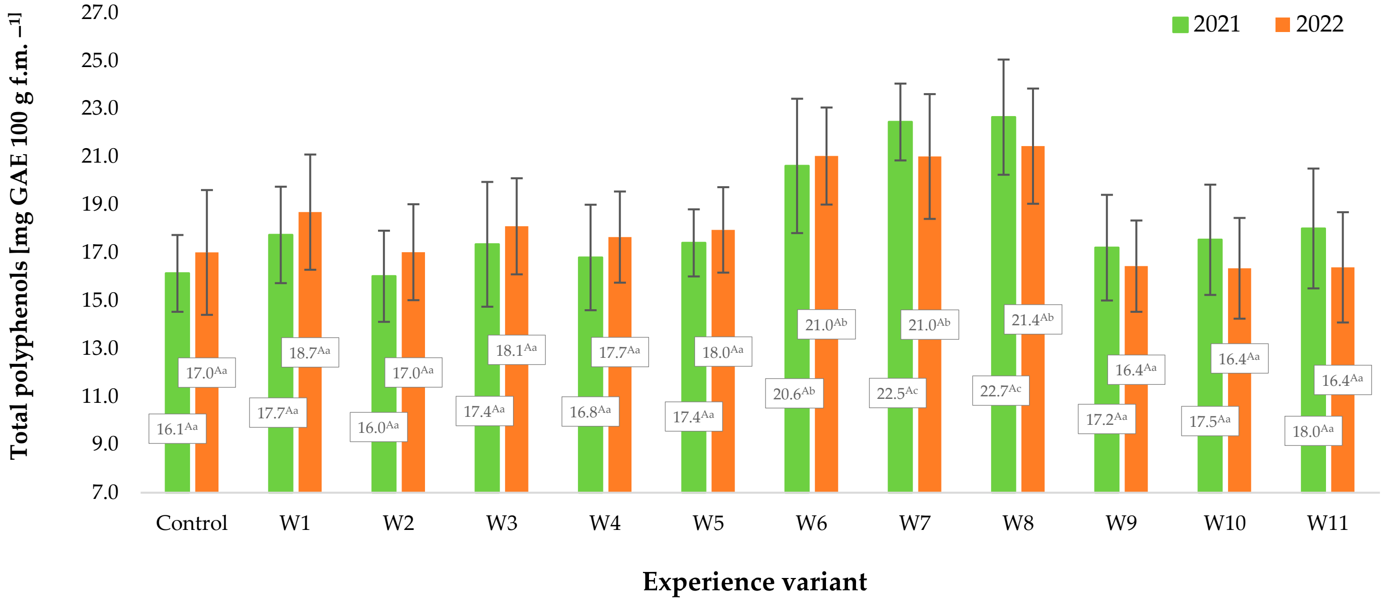

Figure 2. The influence of treatments supporting plant protection during the growing season on the total polyphenol content in fruit in the years of research. Note: Different lowercase letters indicate significant differences between individual experimental variants in a given year, while different uppercase letters indicate significant differences between the same experimental variants in the examined years; significant differences at $p < 0.05$.

The group of antioxidants includes many groups of compounds that shape the total antioxidant potential of raw materials [52]. It was shown that a significant increase in this potential in tomato fruits was caused by the use of gaseous O_3 only. Ozone, as a gaseous substance, has the highest ability to penetrate plant tissues among the factors used, which comprehensively influenced the modification of the biosynthesis of the compounds that contribute to the total antioxidant potential of tomato fruits. From analyzing the obtained test results (Figures 2 and 3), it seems that a state of controlled oxidative stress was achieved in the case of the use of gaseous ozone (Figure 2, W3–W5) and the combined method with the use of solutions with a lower concentration of H_2O_2 (Figure 1, W6–W8). In other cases, the number of oxidizing agents used caused a reaction that resulted in a reduction or no significant increase in phenolic compounds compared to the control and others, shaping the total antioxidant potential.

Vitamin C is a substance belonging to the group of small molecular antioxidants, the biosynthesis of which in plants can be influenced by many factors. When the ozonation process is used, its content changes significantly depending on the conditions used (O_3 concentration and exposure time). Plant raw materials which have lost the ability to metabolize have been subjected to the ozonation process, and in most cases, after the process, they show a significantly lower content of this substance [53,54]. However, when ozonated raw materials have the ability to metabolize, this process may intensify the biosynthesis of vitamin C. A similar effect may be demonstrated by H_2O_2, the solutions of which have a strong oxidizing effect, and its decomposition products are even more active. The analysis of the obtained results (Figure 4) on the influence of the applied factors supporting the protection of tomato plants showed that in most variants, an increase in the content of vitamin C in tomato fruits was observed. However, this increase depended on the ozonation process conditions used and the H_2O_2 concentration.

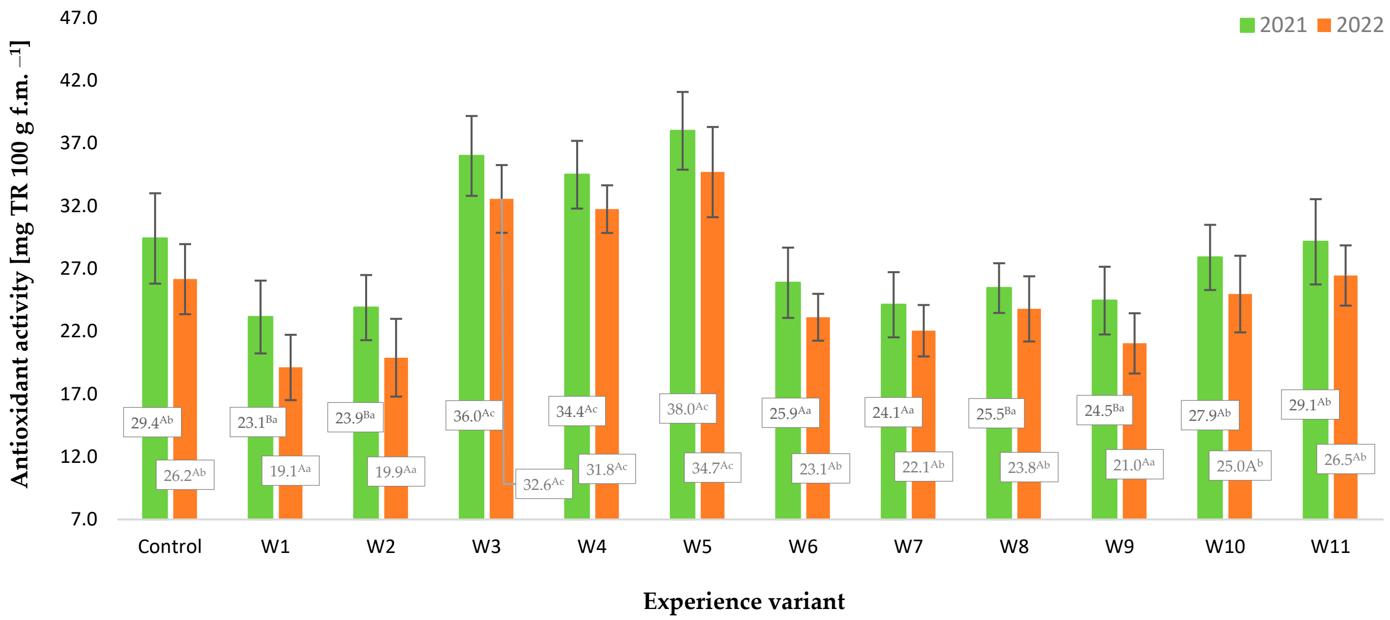

Figure 3. The impact of treatments supporting plant protection during the growing season on the total antioxidant potential of fruit in the years of research. Note: Different lowercase letters indicate significant differences between individual experimental variants in a given year, while different uppercase letters indicate significant differences between the same experimental variants in the examined years; significant differences at $p < 0.05$.

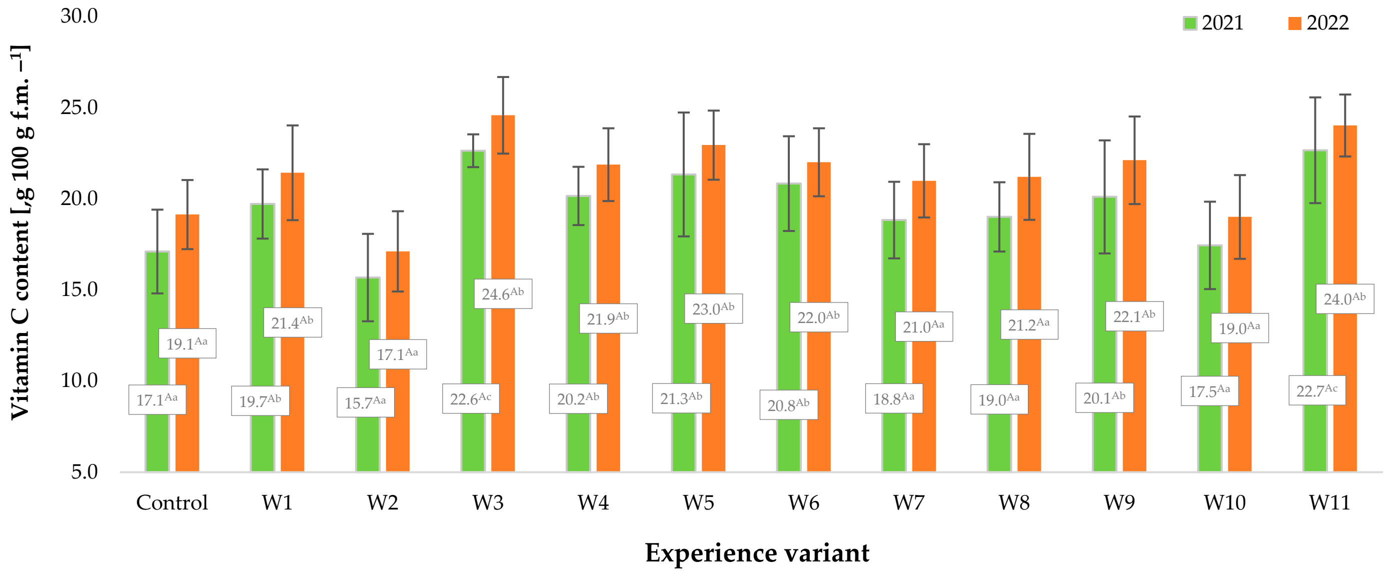

Figure 4. The influence of treatments supporting plant protection during the growing season on the vitamin C content in fruit in the years of research. Note: Different lowercase letters indicate significant differences between individual experimental variants in a given year, while different uppercase letters indicate significant differences between the same experimental variants in the years studied; significant differences at $p < 0.05$.

Vitamin C is a one of many compounds which form antioxidant potential, the biosynthesis of which in plants can be influenced by many factors. When the ozonation process is used, its content changes significantly depending on the conditions used (O_3 concentration and exposure time) [55].

Fruit-bearing plants subjected to the ozonation process may undergo modifications that delay the fruit ripening process. The mechanism of this action may be complex, but decomposition or inhibition of the action of ethylene is usually observed [56]. A measure of this effect can be measuring the mechanical properties of the fruit.

The average values of the destructive force in the process of the uniaxial compression of tomato fruits after fumigation with ozone gas and hydrogen peroxide spraying with various combinations compared to the control are shown in Figure 5. Both in the first and second year of the study only for the variant treated with ozone gas at a dose of 2 ppm for 3 min, there was a significant increase in destructive force compared to the control. For the remaining combinations of variable factors used during the experiment, no positive impact was noted compared to the control sample.

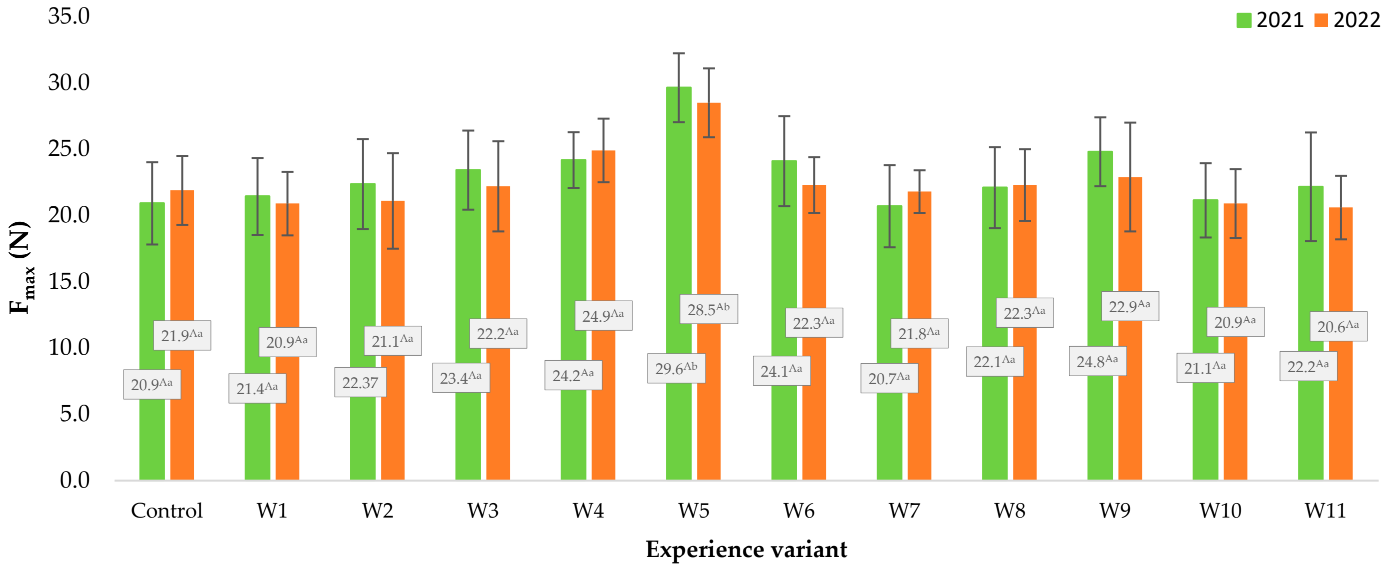

Figure 5. The influence of treatments supporting plant protection during the growing season on the value of the destructive force when pressing tomato fruits in the years of research. Note: Different lowercase letters indicate significant differences between individual experimental variants in a given year, while different uppercase letters indicate significant differences between the same experimental variants in the examined years; significant differences at $p < 0.05$.

In the case of sea buckthorn fruit, an appropriately selected dose of ozone (10 ppm for 15 min) increased the fruit's resistance to mechanical damage [57]. Red currant fruits ozonated with a dose of 10 ppm 30 min after 15 days of storage were characterized by greater resistance to mechanical damage compared to the control fruits [58].

Both in field conditions and under cover, when growing *Solanum lycopersicum*, there is a need to provide protection, mainly against fungal pathogens [59]. Even though growing tomatoes under cover protects the plants against direct exposure to rain, which increases the infection of plants with diseases, especially fungal ones [60], it is necessary to protect them with plant protection products [61]. It should be noted that the use of pesticides affects the quality of the produced tomato fruit, mainly by generating residues [62]. Moreover, the applicable legal regulations related to the "European Green Deal" require the search for alternative methods of protecting plants against diseases and pests, which will allow for limiting the use of fungicides while maintaining the quality and quantity of the produced raw material [63]. One of such potential methods may be the use of the plant ozonation process using ozone gas. Ozone as an allotropic form of oxygen with high oxidizing potential and strong fungicidal and bactericidal properties can be an effective factor limiting the occurrence of fungal diseases in plant cultivation. Its greatest advantage is high antibacterial effectiveness with no chemical residues in the produced plant raw materials. It should be noted that standard plant protection products, by creating residues, also extend the action of the product for a certain period of time after its foliar application. The proposed methodological approach also allows for the use of spraying with a hydrogen peroxide solution, the half-life of which is much longer than in the case of the ozonation treatment carried out under controlled conditions. Moreover, during its decomposition, atomic oxygen is produced, which also has high antimicrobial effectiveness [64].

In recent years, plasma agriculture has emerged as a promising and innovative field with the potential to evaluate conventional farming practices. The application of plasma-based technologies in agriculture offers new opportunities, but the main barrier is the possibility of the generation of various active species, including reactive oxygen (ROS) and nitrogen species. Some nitrogen derivatives like nitrogen oxides could be converted to nitric acid with potential phytotoxic effects [65].

4. Conclusions

This paper describes the impact of the ozonation process and hydrogen peroxide spraying on tomato plants during two years of research. Research has shown that the selected ozonation doses, 2 ppm 1 for min and 2 ppm for 3 min, and the combination of two variable factors, 2 ppm for 3 min combined with spraying with 3% hydrogen peroxide, influence the increased concentration of vitamin C in tomato fruits. In the case of the total content of the phenolic compounds, it was observed that the ozone dose of 2 ppm for 1.5 min and 2 ppm for 3 min in combination with spraying with hydrogen peroxide at a concentration of 1% significantly increased the concentration of these bioactive compounds. However, the antioxidant potential of tomato fruit increased only after each dose of the ozonation process compared to the control. The mechanical properties of the collected fruits were also examined. The research shows that the use of variable factors in most cases does not have a significant impact on the mechanical properties of the tested fruits. Only fruits harvested from ozonated plants showed significantly higher resistance to damage, which indicates a slower ripening process.

The research results show that ozonation and hydrogen peroxide can be effectively used to improve the parameters determining the quality of tomato fruit grown under cover, and after confirming their effectiveness, they can constitute an alternative to fungicides used in tomato cultivation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su16083481/s1. References [66–68] are citied in the Supplementary Materials.

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