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YEAST SOLUTION SPRAY AND OZONE GAS FUMIGATION AS METHODS OF PROTECTING TOMATO PLANTS AGAINST DISEASES

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ABSTRACT

The aim of the study was to determine the effect of ozone fumigation and spraying with microorganisms, i.e. yeast, on selected physiological parameters, and the extent of disease infection of tomato plants. Tomato plants were grown in the ground under covers. Throughout the 2021 and 2022 growing season, the plants were exposed to variable factors, i.e. fumigation with gaseous ozone at a dose of 2 ppm for 1 minute and spraying with yeast. After ozonation and spraying with yeast, physiological measurements were made on tomato plants, such as: selected parameters of chlorophyll fluorescence (maximum quantum yield of PSII photochemistry, maximum quantum yield of primary photochemistry) and gas exchange parameters (transpiration rate, stomatal conductivity and intercellular CO_2 concentration). In addition, the content of chlorophyll using the SPAD method as well as plant infection by diseases was determined. Compared to the control sample, ozonation resulted in a slight decrease in the physiological parameters of tomato plants. In addition, no visible leaf damage was observed after the ozone gas fumigation process. However, the use of microorganisms did not significantly change the examined physiological parameters. Both after the application of gaseous ozone and microorganisms, a significant impact of the tested variable factors was found on reducing the infection of tomato plants by diseases such as: tomato late blight, tomato alternariosis, gray mold, bacterial canker of tomato and bacterial spot of tomato.

Introduction

Tomatoes (*Solanum lycopersicum* L.) are the second most important vegetables in the world after potatoes (Faostat, 2019). Tomato fruits are rich in bioactive compounds that have a beneficial effect on organisms. They contain vitamins and minerals. In addition, these are low-calorie fruits and protect the human body against the adverse effects of free radicals which cause development of many diseases, such as: atherosclerosis, diabetes, cardiovascular diseases and cancer (Sharoni et al, 2016; Mordente et al, 2011). Tomatoes are susceptible to biotic factors, including fungal, viral and bacterial pathogens. The most important diseases causing necrotic changes on tomato plants include tomato blight, tomato alternariosis, tomato brown rot, gray mold, bacterial canker of tomato and bacterial spot of tomato. Among the diseases mentioned above, tomato blight causes the greatest damage to tomato cultivation (Kumar et al., 2018).

The microorganism responsible for tomato blight is *Phytophthora infestans.* This fungus-like microorganism is very aggressive and can potentially infect all parts of plants, causing them to die quickly. This disease attacks plants of various *Solanum* species, including peppers, petunias and eggplants. However, it is best known for destroying tomato and potato plants (Leesutthiphonchai et al., 2018). *Phytophthora infestans* is one of the microorganisms that threaten the world's food security. Considering the history of plant pathologies and the huge yield losses caused by this microorganism, the occurrence of tomato late blight should be closely monitored and effectively destroyed (Yuen et al., 2021). Symptoms of tomato blight appear on all above-ground parts of tomatoes. Infected leaves usually have brown spots of dead tissue surrounded by a pale green or gray border. Lesions on the stem and petioles are brown and usually have an unclear shape. Discoloration may also occur on the flowers, causing them to fall. Symptoms on tomato fruit include mottled, often golden or dark brown, sunken surfaces (Kumar et al., 2018).

Soil or water pollution, climate change or other anthropogenic effects can cause severe abiotic or biotic stress to both crop plants and natural vegetation. Plants are often exposed to various abiotic stress situations. Plants have developed several defense mechanisms in order to overcome such stress and return to basic metabolism (Uddling et al, 2018; Poorter et al., 2021). Plants respond to stress-induced stimuli with marked changes related to their development and physiology. In this context, many processes such as photosynthesis and gas exchange (Menezes-Silva et al., 2017; Bryant et al., 2021), enzyme activity (Makarevitch et al., 2015) or antioxidant profile (Choudhury et al., 2017) may change during stress.

One of the factors classified as abiotic stresses is tropospheric ozone (O_3), which occurs in the environment mainly due to human activity. As a result of economic development, there has been an increase in chronic atmospheric pollution with ozone, which is formed as a result of photochemical reactions (Grulke and Heath, 2020). The responses of crop plants to such exposures are well known, especially considering the effect of ozone on their growth and development (Lesser et al., 1990). Research results show that ozone is considered a stress factor for plants and causes visible damage, reducing the activity of the RuBisCO enzyme, the content of chlorophyll and the intensity of photosynthesis. In addition, as a result of the ozone action, there are changes in stomatal conductivity, carbon (C) allocation of of crop plants. The effect of gaseous ozone on the yield and quality was also noted (Pleijel et al., 2018).

In modern agriculture, natural microorganisms are increasingly used in various ways. Beneficial microorganisms used in agricultural and horticultural crops support plant growth through various mechanisms such as: improving the availability of nutrients; root development; reducing the level of toxic compounds in the soil; improving resistance to biotic and abiotic stresses (Glick and Gamalero et al., 2021). The best example are plants growing in deserts and salty and polluted soils, which, thanks to microorganisms, can overcome abiotic stresses (Xu et al, 2020; Chevin et al., 2017). More and more scientific reports indicate that some plant diseases occur less frequently in some plants, which leads to the hypothesis that disease reduction may be caused by living microorganisms (Lutz et al., 2020; Kohl et al., 2019). Plants completely free of microorganisms are a minority, and in recent years, new beneficial microorganisms that can be used in agricultural production have been identified (Chandran et al., 2021). The best-known and oldest beneficial microorganisms are Rhizobia. These microorganisms colonize the roots of legumes and provide them with nitrogen. Recent years have been a continuous search for new microorganisms and learning about the molecular mechanisms regulating plant-microorganism interactions (Berg et al., 2021). The number of recognized microorganisms interacting with plants, including beneficial ones, is large and constantly growing (Maitra et al., 2022). Beneficial microorganisms within soil bacteria stimulate plant growth and health through various mechanisms (Glick and Gamalero, 2021) such as: production of plant hormones; nitrogen fixation; inorganic phosphate solubilization and organic phosphate mineralization; antagonism against pathogens by production of antibiotics, enzymes and fungicidal compounds; and competition with harmful microorganisms that cause diseases in crops (Di Benedetto et al., 2017). Microorganisms, including yeast, are very often used in tomato cultivation. These microorganisms are used to water tomato plants, which supports the development of the plant root system, and at the same time facilitates the absorption of water and nutrients (Pokluda et al., 2021).

There are quantitative indicators of plant health that can be measured just after or during abiotic and biotic stresses. Several physiological parameters are used for this purpose, such as the rate of stomatal opening, the maximum quantum yield of photosystem II F_v/F_m, intercellular CO_2 concentration, transpiration rate or stomatal conductivity. These parameters change quickly in plants that are unable to cope with unfavorable environmental conditions (Radha et al., 2023). Using these non-invasive techniques, one can easily determine the strength of the impact of a given stress factor on crops or plants growing naturally in the environment.

The aim of the study was to demonstrate the effect of gaseous ozone and yeast on the parameters of chlorophyll fluorescence, gas exchange, chlorophyll content using the SPAD method and infection by diseases of tomato plants grown under cover. It was hypothesized that an appropriate dose of gaseous ozone and yeast spraying would have a positive effect on the physiological parameters of tomato plants and the reduction of infection by pathogens.

Materials and Methods

Plant Material and Growth Conditions

Plant material in a field experiment under cover

A two-year field experiment (year 2021 is marked with the symbol "I"; year 2022 is marked with the symbol "II") was carried out on tomatoes of the Remiz F1 variety (*Solanum lycopersicum*). This variety is predisposed to growing in greenhouses and plastic tunnels. Plants of this variety are tall and strongly branching with stiff stems. The tested cultivar is characterized by high fertility, early maturity and resistance to tobacco mosaic virus (T_m). The fruits are slightly flattened, nicely staining an intense red color, weighing 80-100 g.

The field experiment under cover was assumed for four variants:

– control - no variable factors were used,
– ozone - fumigation with gaseous ozone was carried out,
– ozone + yeast - fumigation with gaseous ozone and yeast spraying were carried out,
– yeast - only spraying with yeast was used.

Growth Conditions

In the experiment in both 2021 and 2022, the fore crop for tomatoes was onion. After harvesting the fore crop, soil samples were taken from the field surface with an Egner's stick. Based on the soil analysis, fertilization doses appropriate for tomato plants were determined. The tested soil pH was suitable for tomato cultivation and amounted to 6.1. In autumn, phosphorus-potassium fertilization was applied, and plowing was performed. Pre-sowing cultivation was carried out in spring. Then foil tunnels were set up. The gardening foil used in the experiment was made of polypropylene. Tomato seedlings were planted at the beginning of May in each year of the study. Plants were planted in rows at distance of 50 cm, and a 100 cm wide passage was left between the rows (Fig. 1). Large-nutrient fertilization (NPK Mg Ca) was applied from the 3rd week after planting out the seedlings. During the period of intensive flowering and yielding, spraying containing microelements, e.g. boron, copper and iron, was applied. Tomato plants were grown "on one shoot", i.e. the growing side shoots were regularly removed to concentrate the plant's energy and development on fruit growth. In addition, excess leaves were removed to accelerate fruit ripening and improve plant health, i.e. better ventilation and improvement of light conditions in the lower parts of plants. In mid-August, the top of the main shoot above the 3 leaves above the last developed inflorescence was removed from the plants.

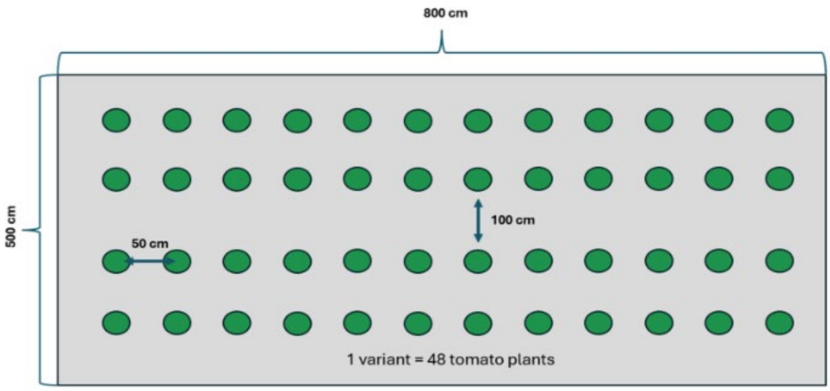


Figure 1. Experimental diagram for each variant (green circles indicate plants)

Experimental treatments used during plant vegetation

Ozonation of tomatoes plant

The research work was preceded by an experimental determination of the plant tolerance threshold for the ozonation treatment. For this purpose, plants of the same variety from other batches were exposed to ozone concentrations of 2, 5, 10 and 50 ppm for 1, 3 and 5 minutes. As a result, *Solanum lycopersicum* plants under cover conditions were periodically fumigated with ozone gas at a concentration of 2 ppm for 1 minute, because no phytotoxic effects were observed in these conditions. A network of channels supplying ozone to each tomato plant was made in the foil tunnel. The foil tunnel was closed, and the hose from the Korona L5 ozone generator (Korona Science and Implementation Laboratory, Piotrków Trybunalski) was connected to the network by means of flexible connections. In the process of fumigation of tomato plants, the concentration of gaseous ozone was measured with the ozone detector 106 M 2B Technologies in the measurement range of 0-1000 ppm. In order to obtain reliable results, the experiment 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 with the aid of air exchange by means of mechanical ventilation, completely removing ozone within 10 seconds.

Yeast spraying

Solanum lycopersicum plants of the "ozone+ yeast" and "yeast" variants were sprayed with 2% yeast saccharomyces cerevisiae (20 g of yeast per 1 l of water) during the growing season under cover. Each time after the ozonation process of the "ozone + yeast" variant, tomato plants were sprayed with yeast. A handheld backpack sprayer was used to perform the treatment. Depending on the vegetation period, 10 ml to 200 ml of spray liquid was used for one tomato plant. The yeast solution was prepared immediately before spraying. Pressed baker's yeast (*Saccharomyces cerevisiae*), dedicated to the food industry (Lallemand, Poland) was the innoculum.

Physiological parameters

During the plant vegetation period, selected physiological parameters were measured once a month for each variant of the experiment. The measurements were carried out with the use of a fluorine meter - the OS30p+ stress meter (Geomor-Technik Sp. z o.o. Szczecin, Poland) and the LCi T compact system for measuring gas exchange - the intensity of photosynthesis (Geomor-Technik Sp. z o.o. Szczecin, Poland).

In addition, in order to determine the impact of the applied variable factors on the degree of infection with any diseases, observations were carried out once a week using a 9-point scale of leaf surface infection: 1° - trace infection, healthy plants, 2° - 1−2%; 3° - 3-4%; 4° - 5-9%; 5° - 10-14%; 6° - 15-24%; 7° - 25-39%; 8° - 40-59%; 9° - 60-100%. Then, the leaf infection index was calculated according to the formula:

$$\text{Disease infestation index} = \frac{\Sigma\,(\mathrm{P\,x\,W})}{\mathrm{i\,x\,n}}\; x\,100 \qquad \dots (1)$$

Σ (P × W) – the sum of the multiplication of the number of infected leaves (P) in a given infestation value (W), i − the highest infestation class, n − the number of all assessed leaves (Rachoń et al., 2018).

Statistical analysis

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

Results and discussion

Measurement of physiological properties of tomatoes plants

Chlorophyll fluorescence was measured 4 times during the growing season of tomato plants, from June to September every year of experience. Figure 2A shows the maximum photochemical efficiency of PSII (F_v/F_m) depending on the applied variable factors. In the first measurement date, which was carried out in June, similar values of the examined parameter were observed for the analyzed variants of the experiment, and they ranged from 0.816 to 0.824 in first year and from 0.817 to 0.820 in the second year. The second measurement was carried out in July and F_v/F_m values were recorded at the level of 0.793 for the control (1st year) and 0.783 for the control variant (2nd year). The ozonated variant and the variant sprayed with yeast and ozonated were characterized by similar values of the tested parameter both in the first and second year of the study. The results for the yeast sprayed variant were similar to those of the control. On the third measurement date, performed in August, the highest value of the F_v/F_m ratio was found in the control sample (0.827 - 1st year, 0.825 - 2nd year). However, for the variants in which variable factors were used, lower values of the tested parameter were recorded, ranging from 0.814 to 0.817 for the first year of research and from 0.811 to 0.818 for the second year of research. The last measurement (IV) was performed in September. The highest value of the analyzed indicator (regardless of the year of research) was observed for the variant of plants sprayed with ozonation and yeast, and the lowest for the control sample. Analogous results after the use of variable agents such as ozone gas and yeast spray were reported for the maximum quantum yield of primary photochemistry (F_v/F_0) (Fig. 2B).

Chlorophyll fluorescence parameters

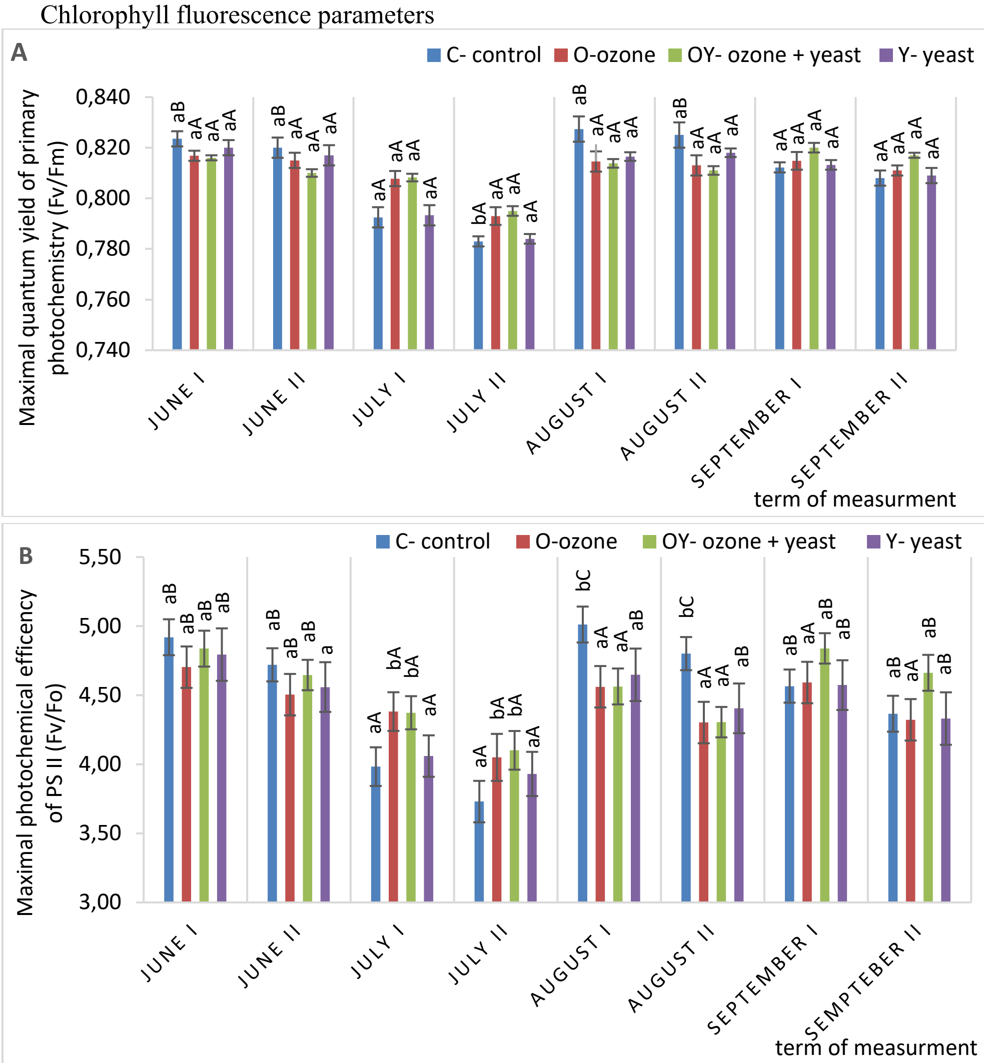


Figure 2. Average values of chlorophyll fluorescence parameters - maximal photochemical efficiency of PSII (F_v/F_m) (A) and maximum quantum yield of primary photochemistry (F_v/F_o) (B) of tomato plants. Significance level $p < 0.05$. The year 2021 is marked with the Roman

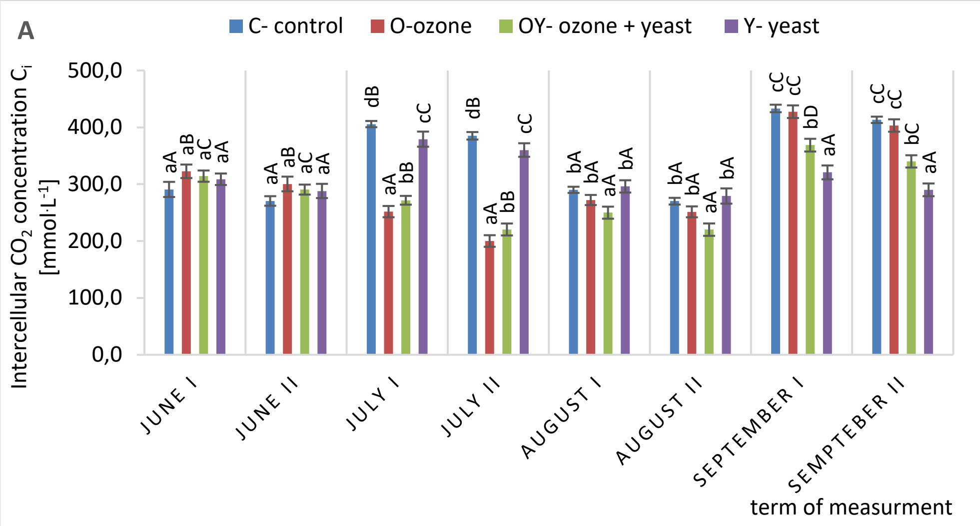
numeral I and the year 2022 with the number II. Lowercase letters indicate differences between variants with the test date. Capital letters mark the differences between test dates.

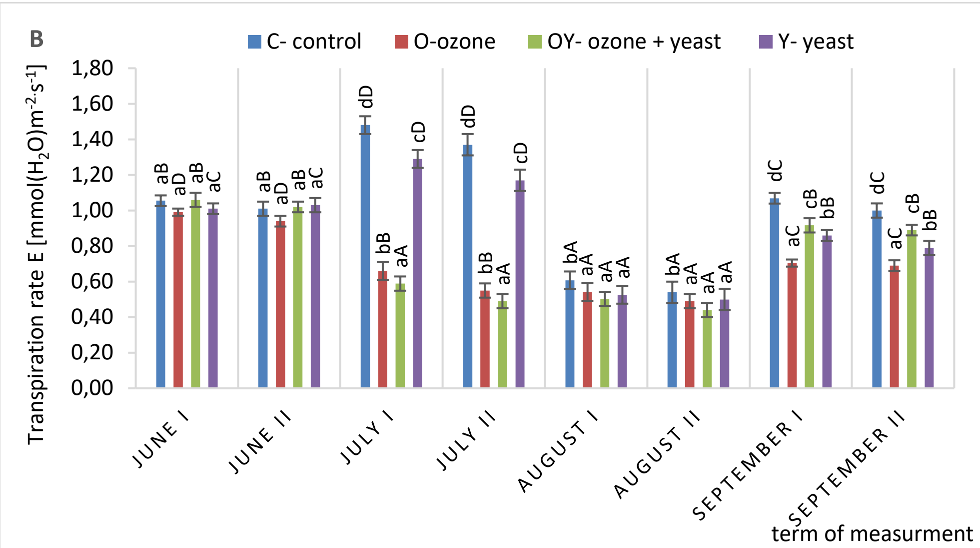
The studied parameters F_v/F_m and F_v/F_0 contain the same information, but the Fv/Fm index is more sensitive and reflects the potential activity of PSII (Guidi et al., 2019). The maximum quantum efficiency of the photosystem II (F_v/F_m) is an indicator that is commonly used to determine the state of photosynthesis in plants. By performing the F_v/F_m test of plants, it is easy to determine whether the applied biotic and abiotic variable factors have a stressful effect on the tested plants. In addition, the occurring stress in the growing environment of the selected plants translates into a decrease in the value of the maximum quantum efficiency of the photosystem (Wu et al., 2023). The F_v/F_m parameter is recognized as a reliable measure of the photochemical activity of the photosynthetic apparatus. Under optimal plant growth conditions, its value should be around 0.850 (Angelini et al., 2001). The appropriate F_v/F_m range for many plant species is approximately 0.790 to 0.840, with lower values indicating greater stress on plants (Kalaji et al., 2017). The results of the maximum photochemical efficiency of PS II for the plants of the experiment on which variable factors in the form of ozone and yeast were used, oscillating in the range from 0.793 to 0.820 and testify to the lack of negative impact of the applied dose of ozone, yeast and combined techniques on tomato plants. The values of the studied parameters of chlorophyll fluorescence as a result of the use of ozonation or yeast spraying during the vegetation period are slightly reduced. It is worth noting that scientific research shows that if the value of the Fv/Fm parameter reaches 0.2-0.3, it means stress and inhibition of the photosynthesis process. Strong stress factors cause irreversible changes in the structure of PSII (Kalaji et al., 2014a; Kalaji et al., 2014b).

In general, ozone is considered a stress factor that can significantly reduce the intensity of photosynthesis, hinder the photosynthetic electron transfer chain and thus reduce the fluorescence parameters of chlorophyll. In addition, the increase in ozone concentration limits the light energy used in photochemical reactions of plants (Wan et al., 2010; Hoshika et al., 2012). Paramater F_v/F_m is reduced under the influence of gaseous ozone, in particular by reduction of the maximum photosynthesis (F_m) (Flowers et al., 2007). The results of F_v/F_m and F_v/F_o (fig. 2 A, B) for the ozone doses used in the field experiment were selected for tomato plants in such a way as not to cause phytotoxic effects, but only to disinfect the surfaces of cultivated plants from pathogens.

Long exposure of forest ecosystems to ozone present in the atmosphere causes a decrease in chlorophyll fluorescence parameters of many tree species. The F_v/F_m values of individual tree species decreased by up to 50% under the influence of long exposure to gaseous ozone (Cao et al., 2012; Liang et al., 2010). Taking into account the literature reports given above, in the present experiment, a high dose of ozone was used, effective in reducing microflora, which had a short-term effect on tomato plants. The results of the tests presented in the experiment show that the ozone dose of 2 ppm had no phytotoxic effect and definitely reduced the microbial load.

Gas Exchange Parameters





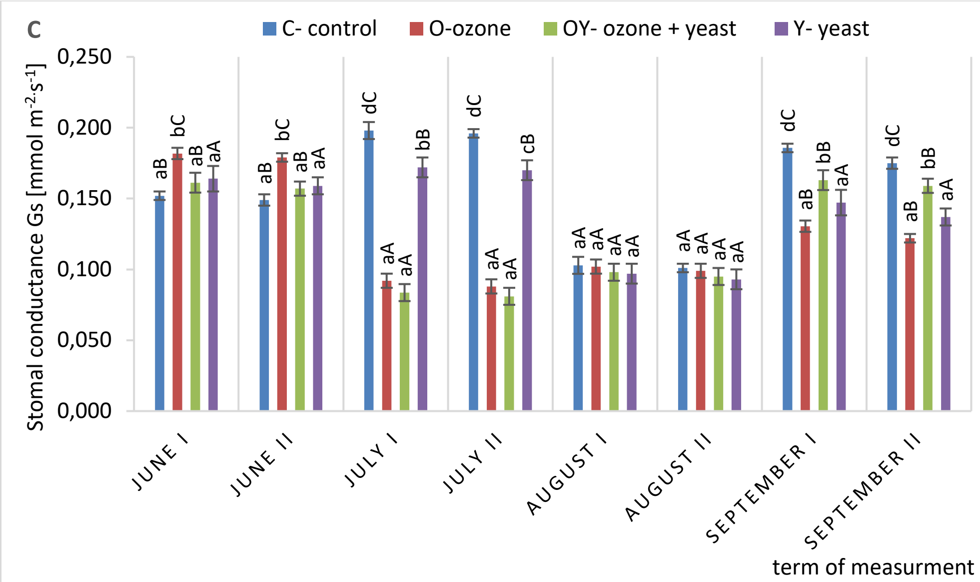


Figure 3. Mean values of gas exchange parameters: intercellular CO_2 concentration (Ci) (A) transpiration rate (E) (B) and stomatal conductance (g_s) (C) of tomato plants. The year 2021 is marked with the Roman numeral I and the year 2022 with the number II. Significance level $p < 0.05$. Lowercase letters indicate differences between variants with the test date. Capital letters mark the differences between test dates

The physiological processes of plants, both cultivated and growing in the natural environment, are often disturbed. There are several stress factors that adversely affect these processes, limiting growth and development. Ozone is one of the stress factors . As a result of the progressing urbanization process, the demand for energy is growing, which in turn leads to the emission of large amounts of pollutants and an increase in the concentration of ground-level ozone at the rate of 0.5-2.5% per year (Monks et al., 2015; Sahu et al., 2021). However, these concentrations rarely exceed several hundred ppm, but they are harmful due to the chronic nature of the phenomenon.

On the first measurement date, carried out in June for 2021 and 2022, there was no negative impact of the variable factors applied in the experiment on the values of the tested physiological parameters (G_s, C_i and E). For the next measurement performed in July, it was observed that gaseous ozone significantly reduced the value of all tested parameters compared to the control in both the first and second year of the study. A slight decrease in the values of the tested parameters was also found compared to the control sample for plants sprayed with yeast. In the case of measurements performed in August 2021 and 2022, the results of the tested physiological parameters did not differ significantly after the use of variable factors. Based on the measurement results carried out in September of both years of the study, it was found that the lowest E and G_s values were recorded for ozonated plants regardless of the year of the experiment. Additionally, slightly higher values of these parameters were recorded compared to ozonated plants after yeast spraying and ozonation and yeast spraying. However, the highest E and G_s values were recorded for control plants. In the case of the C_i parameter, the values of this indicator for control and ozonated plants were at a similar level regardless of the year of research. Lower values of this indicator were recorded for plants of the variant sprayed with yeast in each year of the study.

Gaseous ozone in chronic exposure as a stress factor contributes to the aging of leaves and the reduction of the number of stomata (Agathoklfous et al.,2016) and limits carbon sequestration (Agathoklfous et al.,2015). As a result of the increase in the concentration of ozone in the atmosphere, the stomata of plants close. It is a way of plant defense against the penetration of ozone into the plant (Paoletii et al., 2005). In addition, when the stomata are closed as a result of ozone-induced stress, the rate of photosynthesis also decreases due to the reduction of CO_2 uptake by the plant (Wang et al., 2016a; Wang et al., 2016b; Li et al., 2018). As a result of the penetration of O_3 through the stomata into the cellular space, the conductivity of the mesophyll decreases, which translates into a decrease in the intensity of photosynthesis in plants (Xu et al., 2019). In addition, it was found that the photosynthesis rate of Metasequoia glyptostroboides significantly decreases when the ozone concentration increases (Feng et al., 2008). Plants that are chronically exposed to ozone as a result of the limited photosynthesis are characterized by lower biomass gains (Li et al., 2019). In addition, the increased ozone concentration reduces the intensity of transpiration, rate of photosynthesis and efficiency of water use by forest ecosystems (Chen et al., 2022).

The results of the study of selected gas exchange parameters presented in this article indicate that the intercellular CO_2 concentration (C_i) from July to September for tomato plants on which the variable factor in the form of ozone was applied is slightly lower than the values obtained for the tested parameter of the control sample. Similar relationships were noted for the rate of transpiration (E) and stomatal conductance (g_s). However, it should be noted that the application of ozone at a concentration of 2 ppm to tomato plants for 1 minute reduces gas exchange parameters compared to the control sample, but the decrease in these values does not indicate the phytotoxicity of this dose. The dose of ozone has been selected in such a way as not to damage the plants and, above all, to reduce the occurrence of pathogens.

Relative Chlorophyll Content

During the experiment, measurements of the relative chlorophyll content in tomato plants (SPAD) were made. The results of the first measurement performed in June 2021 and 2022 are equal, and on their basis, there was no significant impact of the variable factors used on the value of the tested parameter. However, based on the results of subsequent measurements, it should be concluded that gaseous ozone reduced the value of the SPAD parameter of tomato plants compared to the control in two years of research. The use of yeast spray did not negatively affect the chlorophyll content in the leaves. The results for this variant of the experiment are similar to the value of the control sample index in each year of the experiment. In turn, for the ozone fumigation + yeast spraying variant, the SPAD results were lower than for the control sample and the variant of plants sprayed with yeast, but higher than for the variant of plants subjected to ozonation. Based on the results obtained, it should be concluded that ozonation slightly reduced the SPAD value, while another variable factor, i.e. yeast spraying, did not worsen the physiological condition of tomato plants compared to control plants (Fig. 4).

The research results presented in this article are identical to the results of the available literature. The increase in ozone concentration is associated with a decrease in the content of chlorophyll in plants. Oxygen molecules reacting with ozone enter chloroplasts through membrane channels (Grulke and Heath, 2020). First of all, the decrease in the content of chlorophyll in the leaves of plants results in the weakening of the photosynthesis process. Ozone penetrating through the stomata reduces the needs of vegetation for light energy (Matyseek et al., 2008). Chen et al., (2022) studied poplar trees and found that the impact of ozone stress reduces the intensity of photosynthesis, which translates into a lower concentration of chlorophyll in plant leaves and leads to lower biomass gains. Bussotti et al., (2011) found that many plants in subtropical areas were destroyed by ozone gas, which significantly reduced the chlorophyll content in their leaves. Liu et al., (2015) reported that the chlorophyll content in Weigela florida, Forsythia suspensa and P. tabulaeformis was reduced after applying fumigation with gaseous ozone. It was related to the decreasing maximum photochemical yield of plants as a result of the use of the stress factor ozone. Guo et al., (2019) studied 13 species of trees from southern China and found that their physiological parameters changed as a result of the application of gaseous ozone. The intensity of photosynthesis, chlorophyll content and stomatal conductivity decreased.

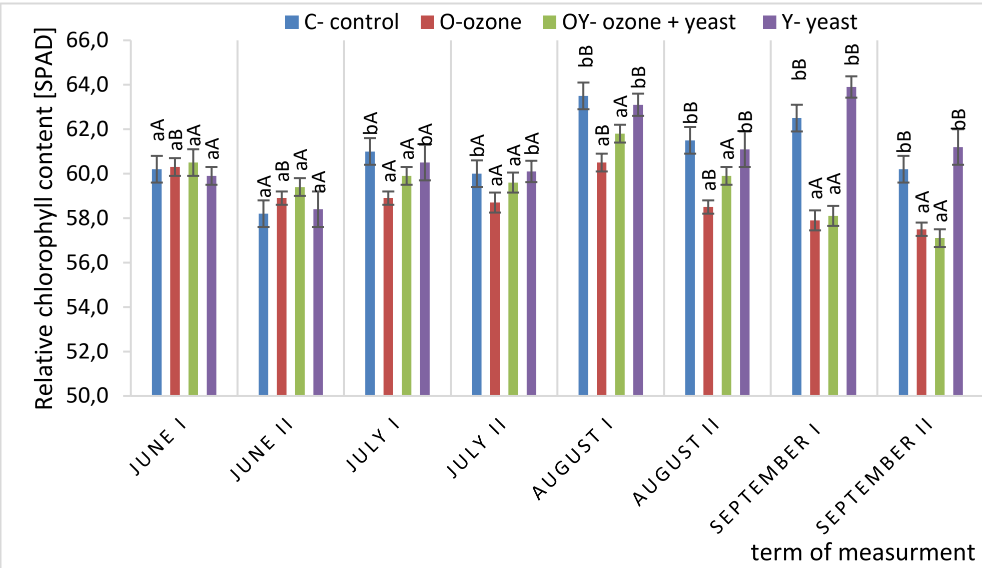


Figure 4. The relative content of chlorophyll (SPAD) in tomatoes leaves. The year 2021 is marked with the Roman numeral I and the year 2022 with the number II. Significance level p <0.05. Lowercase letters indicate differences between variants with the test date. Capital letters differences between test dates

Disease rate determination by diseases

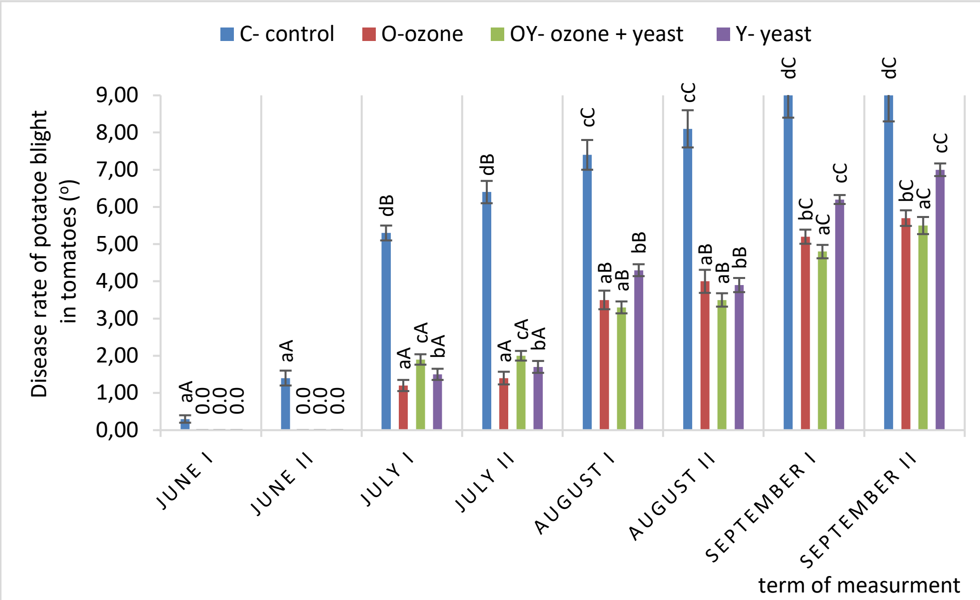


Figure 5. Disease rate of tomato plants by diseases. The year 2021 is marked with the Roman numeral I and the year 2022 with the number II. Significance level p <0.05. Lowercase letters indicate differences between variants with the test date. Capital letters differences between test dates

In June of the first and second year of testing, tomato plants were infected with diseases, including potato blight, in the control sample. The remaining variants were free from the pathogen infection. In July, disease infection was recorded in all tested variants of the experiment. In the control sample, over 10% of infections with pathogenic fungi were found, regardless of the year of testing. In the remaining variants, trace changes of less than 2° were recorded. In August 2021 and 2022, significant disease infection was recorded on control tomato plants, over 40% in the first year of the study and over 45% in the second year of the study. In the variant with ozonated plants and ozonated plants and yeast sprayed, diseases occurred at a rate of 4% in the first year of the study and 6% in the second year. In turn, for the variant of plants sprayed with yeast, disease infection was 7% in the first year of research and 6% in the second year. The highest values of plant infection by pathogens were recorded in September. The control sample was infected 100% in each year of the study, plants sprayed with yeast 23% and 35% in 2021 and 2022, respectively. The variety treated with ozone gas 12% and 14%, respectively, in 2021 and 2022. In turn, tomato plants were ozonated and sprayed with yeast at 17% and 25% in 2021 and 2022, respectively.

Gaseous ozone is used in industry to decontaminate viruses, gram-negative and gram-positive bacteria, spores and fungi (Manousaridis et al., 2005). The use of ozone, due to its short life, is considered a safe method and, above all, does not generate residues. The negative impact on the composition of fruit or vegetables growing on fumigated plants is minimal

(Botondi et al., 2021; Mir et al., 2023; Rahmanti et al., 2022) Thus, gaseous and water-soluble ozone as an antimicrobial agent in food chains is considered "green technology" (Khadre et al., 2001). There are many studies in which the safety of using ozone as a disinfectant for dried food, spices, pistachios, meat or entire production and processing plants has been demonstrated (Akbas and Ozdemir, 2006); Vallone and Stella, 2014) against *Escherichia coli, Bacillus spp.* and Micrococcus. With regard to fungi, ozone effectively inhibits the formation of germ tubes in the pathogenic fungi *C. albicans and A. flavus* (Ouf et al., 2016; Zargaranm et al., 2017). Scientific reports indicate that the fumigation process with gaseous ozone is an innovative method that leads to the degradation of mycotoxins (Afsah-hejri et al., 2020). In addition, O_3 has been used in the control of pepper mild spot virus (Stommel et al., 2021), tomato brown fruit virus (Samarah et al., 2021) and tomato mosaic virus (Paylan et al., 2014). Because the strong oxidizing properties of O_3 eliminate or significantly reduce growth and spore formation (Tzortzakis et al., 2008), the effect of O_3 on infections has often been analyzed during the storage of cereals or fruits to prevent growth and mycotoxin production. An ozone fumigation treatment has been applied to various crops such as cucumber, chickpea, garlic and grapevine (Botondi et al., 2015; De Santis et al., 2021); as well as cereals, i.e. wheat (Sarooei et al., 2019), barley (Piacentini et al., 2017) and rice (Savi et al., 2020). The usefulness of the ozonation process for protecting potato plants against pathogens was also initially determined. It has been shown that ozone in large doses has a strong phytotoxic effect, but appropriately selected process conditions provide favorable conditions for plant growth and the concentrations used are effective in combating bacterial and fungal microflora (Szpunar-Krok et al., 2020). Potatoes and tomatoes belong to one family of Solanaceae, so it can be concluded that the results obtained for tomatoes correspond to the observations made for potato plants subjected to the ozonation process.

Conclusions

The controlled ozonation process used during the growing season of tomato plants grown under cover led to a slight decrease in the tested parameters of chlorophyll fluorescence and gas exchange. Additionally, lower values of the relative chlorophyll content were found for plants ozonated with a dose of 2 ppm for 1 minute compared to the control sample. As a result of the application of gaseous ozone to tomato plants, no phytotoxicity was detected in two growing periods. Moreover, there was a significantly lower infection with plant disease pathogens fumigated with ozone compared to the control sample. In the case of ozone fumigation of tomato plants, a significant reduction in disease infection was observed during the study period. A reduction in pathogen infection was achieved in July by 77.7%, in August by 58.4% and in September by 42.3% in the first year of the study compared to the control sample. In the second year of the study, the pathogen infection was reduced by 74.1% in July, 54.1% in August and 34.1% in September compared to the control sample. The second factor used in the field experiment was the spraying of tomato plants with beneficial microorganisms, i.e. yeasts, during their growing season. It was found that cyclic spraying of plants with yeast did not significantly affect the relative content of chlorophyll in tomato leaves. Additionally, after the use of yeast, a slight reduction in gas exchange parameters and chlorophyll fluorescence was noted compared to the control for each year of the study. As in the case of ozone, spraying plants with yeast significantly reduced the invasion of tomato diseases compared to the control for each year of the study. As a result of spraying tomato plants with yeast solution, beneficial effects were obtained in the form of reduction of the infection of the tested plants with pathogens. The proposed methods can be used as an alternative to standard plant protection products, which is particularly important in organic production which requires the use of residue-free methods.

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OPRYSK ROZTWOREM DROŻDŻY I OZONOWANIE JAKO METODY OCHRONY ROŚLIN POMIDORA PRZED CHOROBAMI

Streszczenie. Celem pracy było określenie wpływu ozonowania oraz opryskiwania mikroorganizmami, tj. drożdżami na wybrane parametry fizjologiczne oraz stopień porażenia roślin pomidora przez choroby. Rośliny pomidora uprawiano w gruncie pod osłonami. Przez cały sezon wegetacyjny 2021 i 2022 rośliny poddawano działaniu zmiennych czynników, tj. fumigacja gazowym ozonem w dawce 2 ppm przez 1 minutę oraz oprysk drożdżami. Po ozonowaniu i oprysku drożdżami, dokonano pomiarów fizjologicznych pomidorów, takich jak: wybrane parametry fluorescencji chlorofilu (maksymalna wydajność kwantowa fotochemii PSII, maksymalna wydajność kwantowa fotochemii pierwotnej) oraz parametry wymiany gazowej (szybkość transpiracji, przewodnictwo aparatów szparkowych oraz międzykomórkowa koncentracja CO_2). Ponadto, określono zawartość chlorofilu za pomocą metody SPAD jak również stopień porażenia chorobami. W porównaniu z próbką kontrolną ozonowanie spowodowało nieznaczne obniżenie parametrów fizjologicznych roślin pomidora. Ponadto nie zaobserwowano widocznych uszkodzeń liści po procesie fumigacji gazem ozonowym. Dodatkowo zastosowanie mikroorganizmów nie zmieniło istotnie badanych parametrów fizjologicznych. Zarówno po zastosowaniu gazowego ozonu, jak i mikroorganizmów stwierdzono istotny wpływ badanych czynników zmiennych na ograniczenie porażenia roślin pomidora chorobami takimi jak: zaraza ziemniaka, alternarioza pomidora, szara pleśń, bakteryjny rak pomidora i bakteryjna plamistość pomidora.

Słowa kluczowe: pomidory, ozon, parametry fizjologiczne, saccharomyces cerevisia