Article

THE INFLUENCE OF EXPIRED MEDICINES ON PLANT PHOTOSYNTHESIS PARAMETERS AND CHLOROPHYLL PIGMENTS

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Abstract:
This study examines the influence of expired pharmaceuticals on Brassica oleracea var. Buzău, grown at three different CO_2 concentrations, focusing on photosynthetic parameters and chlorophyll pigment concentrations. Active pharmaceutical ingredients (APIs) and excipients in expired drugs pose significant environmental and public health risks through improper disposal, leading to soil and water contamination. Pharmaceuticals in the soil significantly impact plant photosynthesis, reducing net assimilation rates and stomatal conductance. Additionally, they cause a marked decrease in chlorophyll a and b concentrations due to toxic effects that degrade chlorophyll and inhibit its biosynthesis. However, an increase in zeaxanthin concentration suggests an adaptive response to oxidative stress. While total phenol concentrations remain relatively unaffected, flavonoid levels significantly decline, disrupting secondary plant metabolism. Elevated CO_2 levels can stimulate phenolic compound synthesis, partially offsetting the negative impacts of pharmaceuticals. The antioxidant activity of plants is also compromised, as indicated by altered inhibition percentages due to reduced phenol concentrations essential for soil microbial activity and chemical reactions. These findings highlight the need for understanding the combined effects of pharmaceuticals and elevated CO_2 on plant health and stress the importance of managing soil contamination to protect ecological health and agricultural productivity.

Keywords: *Brassica oleracea var. Buzău*., abiotic stress, photosynthesis, elevated carbon dioxide.

INTRODUCTION

Active pharmaceutical ingredients (APIs) refer to the biologically active constituents of a medication. Additionally, these components may include additional physiologically active chemicals, typically known as excipients. Excipients are added to several drugs to augment absorption and boost solubility (Kayode-Afolayan et al., 2022). Expired drugs pose a significant issue for both the environment and public health (Begum et al., 2021). Improper disposal of medications, such as indiscriminately discarding them in toilets, sinks, or landfills, can pollute soil (Granger and Nicoll, 2014), groundwater (Ashton et al., 2004), and drinking water sources (Kookana et al., 2014). For example, a study conducted in Kabul, Afghanistan, revealed that out of 301 questionnaires, 77.7% of the respondents disposed of expired medicines in household trash (Bashaar et al., 2017). These pollutants have a detrimental impact on plants and animals, disrupting the ecological equilibrium.

The absorption of medications by plants has been proven on several scales, including laboratory trials and real-life agricultural settings (Garduño-Jiménez and Carter, 2024). The lettuce was grown in a hydroponic system with diclofenac at a concentration of 20 mg/L, and the drug was found in the roots at a very low concentration. However, no diclofenac was identified in the leaves of the *Lactuca sativa* (Bigott et al., 2021). In a preliminary investigation in 2013, Carter et al. conducted a controlled experiment involving radish (*Raphanus sativus*) and ryegrass (*Lolium perenne*). The study aimed to understand the absorption behavior of pharmaceuticals when introduced directly into the soil. The researchers observed that both plant species absorbed five of the six tested pharmaceuticals. They noted that the uptake of these pharmaceuticals was significantly influenced by various physicochemical properties, including the distribution coefficient, which affects the compound's solubility and partitioning behavior. Additionally, soil parameters such as the organic carbon content played a crucial role in determining the extent of pharmaceutical uptake (Carter et al., 2014). Medications can adversely affect aquatic creatures by disrupting their hormones and behavior (Kayode-Afolayan et al., 2022). Additionally, they can hinder the normal breakdown of organic matter and the cycling of nutrients in the soil, which can negatively impact soil fertility and the health of plants (Gerke, 2022). Improperly discarded drugs lead to the emergence of antibiotic-resistant bacteria, which poses a significant concern to public health due to the difficulty in treating infections caused by these bacteria, hence increasing the likelihood of illness and death (Ajayi et al., 2024).

On the other hand, the global concentration of carbon dioxide has increased more than ever in the last ten years, reaching an unprecedented level of 426 ppmv in May 2024. This significant rise in carbon dioxide levels is a cause for concern due to its implications for climate change and environmental health. For plants, such an increase in carbon dioxide concentration could have a dual impact. On the one hand, it could enhance the assimilation rate, which refers to the process by which plants convert carbon dioxide into organic compounds during photosynthesis (Jawahar Jothi et al., 2024). This could potentially lead to increased plant growth and productivity (Siddique et al., 2024). However, this positive effect is counterbalanced by potential negative consequences on the cycles of secondary metabolites. Secondary metabolites are organic compounds that are not directly involved in plants' normal growth, development, or reproduction but play crucial roles in plant defense, signaling, and interactions with their environment (Lupitu et al., 2023). Changes in carbon dioxide levels can disrupt these cycles, leading to altered production of secondary metabolites. This disruption can have far-reaching effects on plant health, resilience, and their ability to cope with stressors such as pests, diseases, and environmental changes (Duan et al., 2024).

The *Brassicaceae* family, also known as the mustard family, is one of the most economically significant families of flowering plants. This family comprises 372 genera and 4,060 accepted species, including many important agricultural crops.

This study aimed to analyze the effect of expired drugs on *Brassica oleracea* var *Buzău* grown at three different concentrations of carbon dioxide, on photosynthetic parameters and chlorophyll pigments concentrations.

MATERIALS AND METHODS

Plant material

Brassica oleracea var *Buzău* seeds were planted in plastic pots filled with commercially available garden soil at a depth of 1 cm. The plants were grown under a day/night cycle with temperatures of 25°C during the day and 18°C at night, with a 12-hour daily light regimen. The plants were exposed to three different concentrations of carbon dioxide: 400 ppm, 800 ppm, and 1200 ppm. Experimental analyses were conducted eight weeks after planting. The plants have been watered with one litter of 0.4 g/L active substance solution of the following drugs: Omeprazole (omeprazole), Espumisan (simethicone), Lipantil (fenofibrate), NoSpa (drotaverine chlorhydrate), Dulcolax (bisacodyl), and Ketonal (ketoprofene), expired from 5 months.

Determination of photosynthetic parameters

A GFS-3000 gas exchange device (Heinz Walz GmbH, Effeltrich, Germany) was utilized to analyze the photosynthetic parameters (assimilation rates and stomatal conductance to water vapor) (Copolovici et al., 2017a; Niinemets et al., 2010).

Determination of chlorophyll pigments

High-performance liquid chromatography (HPLC) was employed to identify and quantify plant chlorophyll pigments. Following exposure to various concentrations of carbon dioxide and ozone, leaf samples measuring 4 cm² were collected and extracted with acetone, following the methodology outlined in (Opriş et al., 2013), to determine chlorophylls a and b and other pigments. The analyses were conducted using a high-performance chromatographic system

(NEXERA8030, Shimadzu, Japan) equipped with a diode array detector (DAD).

The determination of total phenolics and flavonoids

The total phenolic content was determined using the Folin-Ciocalteu method, measuring the complex formed at a wavelength of 765 nm. The flavonoid content was determined based on the reaction with aluminum chloride, measuring the yellow product obtained at 434 nm.

Determination of antioxidant capacity

The spectrophotometric method used DPPH (2,2-diphenyl-1-picrylhydrazyl), a stable free radical, to evaluate the antioxidant capacity of plant extracts. The extracted sample was mixed with a DPPH solution and incubated for a specified time, allowing the reaction between antioxidants and DPPH radicals. A spectrophotometer monitored the formation of the complex between antioxidants and DPPH at a wavelength of 517 nm. The antioxidant capacity was determined by measuring the initial and final absorbance of the solution and calculating the percentage of inhibition of DPPH radicals.

RESULTS AND DISCUSSIONS

Photosynthetic parameters

The photosynthetic parameters of control plants were higher than those of plants grown at elevated carbon dioxide concentrations (Figure 1). The assimilation rate decreases in plants treated with various medicines, particularly those grown at high carbon dioxide concentrations. This phenomenon has been specifically observed in plants treated with common pharmaceuticals such as diclofenac (Copolovici et al., 2017b) and paracetamol (Taschina et al., 2017). Our previous study demonstrated that plants from the *Fabaceae* family significantly reduce the assimilation rate when exposed to different concentrations of nonsteroidal anti-inflammatory drugs (NSAIDs) (Taschina et al., 2022). Elevated carbon dioxide concentrations negatively impact stomatal conductance to water vapor. This phenomenon occurs because plants can minimize water loss by reducing maximal stomatal conductance while still maintaining carbon uptake (Beerling and Franks, 2010).

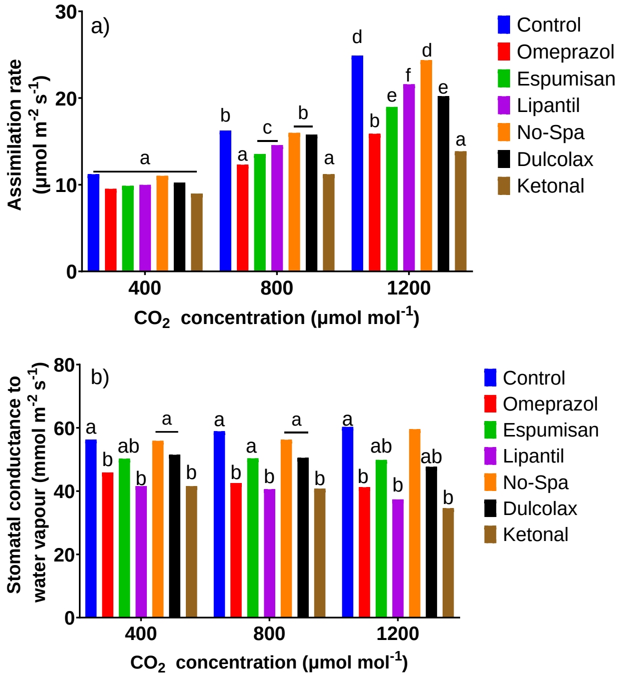


Figure 1. Variation in Assimilation rate (a) and Stomatal conductance to water vapor (b) for *Brassica oleracea* plants grown at high carbon dioxide and treated with 0.4 g medicines. Data sharing different letters are significantly different ($p < 0.05$), while data sharing the same letters are not significantly different ($p > 0.05$).

Additionally, when plants are treated with certain pharmaceuticals, stomatal conductance has a differential response. For instance, treatments with ketonal decrease stomatal conductance, whereas treatments with NoSpa and Dulcolax do not elicit significant changes in this physiological parameter. These findings suggest that the application of these medications can negatively impact the physiological processes of plants, potentially affecting their growth and productivity, especially under elevated carbon dioxide conditions.

The influence of elevated carbon dioxide and drugs on chlorophyll pigments

Chlorophyll pigments, specifically chlorophylls a and b, are adversely affected by the presence of pharmaceuticals in the soil (Figure 2). Conversely, elevated carbon dioxide concentrations increase chlorophyll levels. However, the influence of carbon dioxide is not sufficient to mitigate the adverse effects of the drugs.

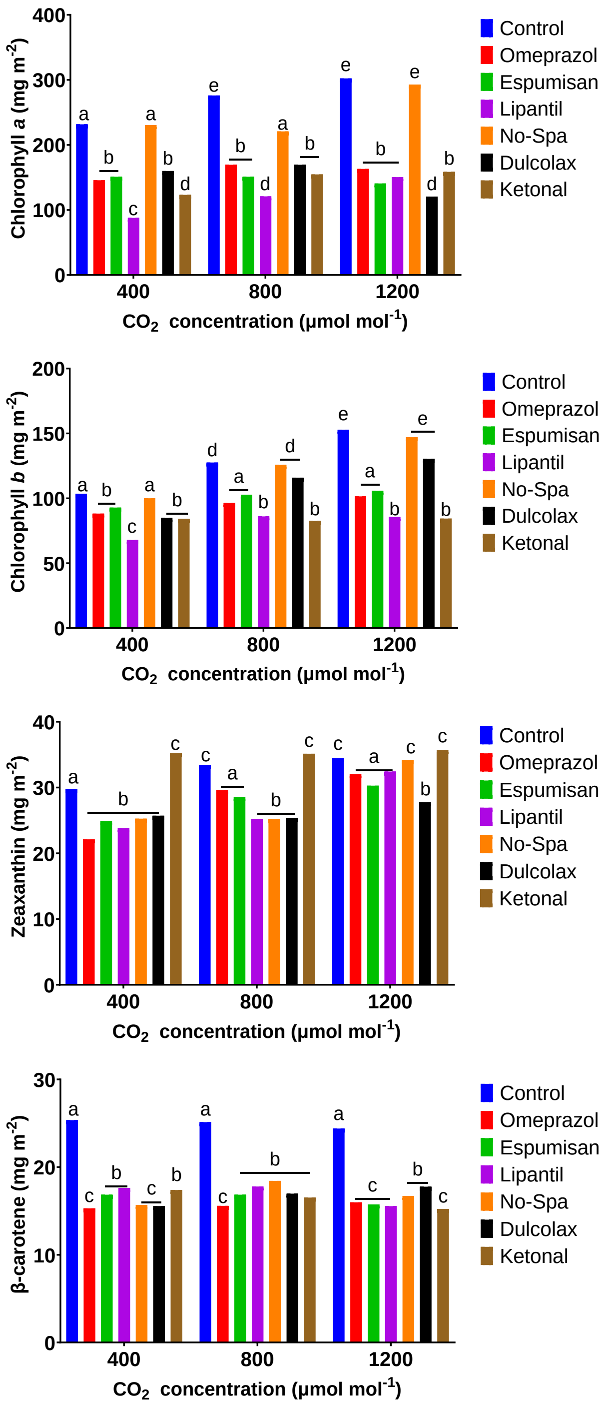
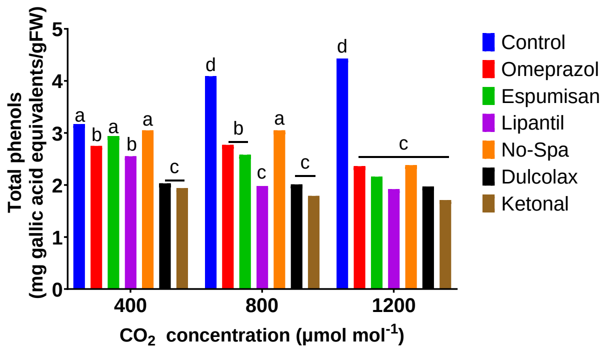


Figure 2. The influence of drugs on chlorophyll pigments in the leaves of plants grown at various CO2 concentrations. Data sharing different letters are significantly different ($p < 0.05$), while data sharing the same letters are not significantly different ($p > 0.05$).

Empirical studies have demonstrated a marked reduction in chlorophyll concentrations within the foliage of plants subjected to various pharmacological treatments (Taschina et al., 2022). This diminution is attributable to the toxicological impacts of these chemical agents on the photosynthetic machinery, resulting in both the degradation of chlorophyll and the inhibition of its biosynthesis (Krupka et al., 2022). Conversely, an upregulation in zeaxanthin concentration has been documented in plants exposed to these pharmaceuticals. Out of the several carotenoids that were found to have varied levels of accumulation, zeaxanthin showed the highest accumulation. This particular metabolite is produced by the oxidation of antheraxanthin by an important enzyme called zeaxanthin epoxidase (Dautermann and Lohr, 2017; Park et al., 2017). This response involves the activation of protective pathways aimed at mitigating oxidative damage. The mechanisms through which pharmaceuticals influence chlorophyll levels encompass interference with both the synthesis and degradation pathways of the pigment, as well as the induction of oxidative stress via the generation of reactive oxygen species (ROS) (Hao et al., 2022). These reactive species precipitate the peroxidation of chloroplast membrane lipids, thereby compromising the structural and functional integrity of chloroplasts.

The influence of drug-induced stress and carbon dioxide on total phenols and flavonoids

While the concentration of total phenols is not strongly affected by the presence of pharmaceuticals, flavonoids show a significant decrease in the presence of these substances (Figure 3). This behavior can be explained by the toxic effect of pharmaceuticals on plants' secondary metabolism, which particularly affects the synthesis and accumulation of flavonoids. On the other hand, the concentrations of total phenols and flavonoids are influenced by the presence or absence of elevated carbon dioxide. Higher concentrations of CO_2 can stimulate the synthesis of phenolic compounds (Ibrahim and Jaafar, 2012), partially compensating for the negative effects of pharmaceuticals on plants.



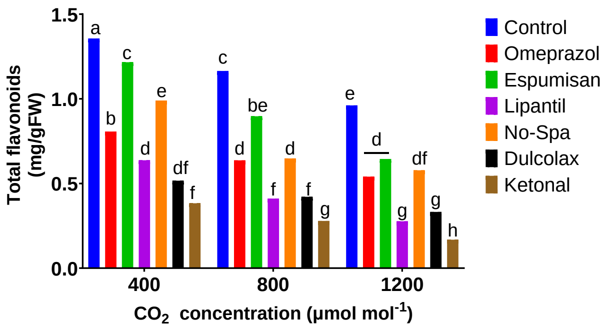


Figure 3. The influence of pharmaceuticals on the concentrations of total phenols and flavonoids in plants grown at different CO_2 concentrations. Data sharing different letters are significantly different ($p < 0.05$), while data sharing the same letters are not significantly different ($p > 0.05$).

Thus, under elevated CO_2 conditions, plants may have a higher antioxidant capacity due to the increased synthesis of phenols and flavonoids.

The influence of pharmaceuticals on the antioxidant activity of plants grown at different CO_2 concentrations

Generally, the percentage of inhibition is altered by the presence of pharmaceuticals in the soil due to decreased phenol concentrations (Figure 4).

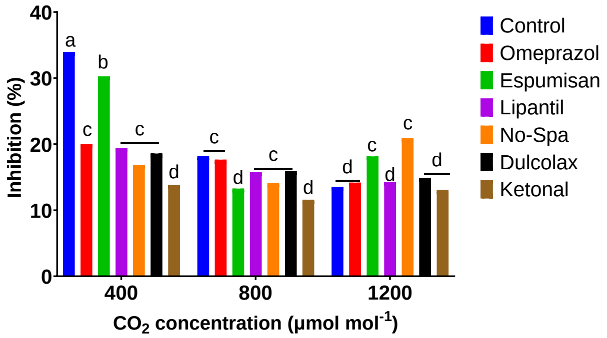


Figure 4. The influence of pharmaceuticals on the inhibition percentage in extracts of plants grown at different CO_2 concentrations. Data sharing different letters are significantly different ($p < 0.05$), while data sharing the same letters are not significantly different ($p > 0.05$).

The presence of pharmaceuticals in the soil can significantly influence various chemical and biological processes. Phenols, aromatic organic compounds, play an essential role in microbial activity and various chemical reactions in the soil. Pharmaceuticals can interact with phenols when introduced into the soil, reducing their concentration.

CONCLUSIONS

The study revealed that the presence of pharmaceuticals in the soil significantly impacts photosynthetic parameters, including net assimilation rate and stomatal conductance. Moreover, pharmaceuticals substantially reduce chlorophyll a and b concentrations in plant leaves, attributed to the toxic effects that degrade chlorophyll and inhibit its biosynthesis. Conversely, an increase in zeaxanthin concentration was observed, suggesting an adaptive response of plants to oxidative stress. While the concentrations of total phenols are not markedly affected by the presence of pharmaceuticals, flavonoids exhibit a significant decline, thereby impacting the secondary metabolism of plants. Elevated CO_2 concentrations have the potential to stimulate the synthesis of phenolic compounds, partially mitigating the adverse effects of pharmaceuticals. The antioxidant activity of plants is also compromised, with the inhibition percentage being modified due to the reduction in phenol concentrations, which are crucial for microbial activity and chemical reactions in the soil. These findings underscore the importance of comprehending the combined effects of pharmaceuticals and elevated carbon dioxide levels on plants. They also emphasize the necessity for meticulous management of soil contamination to safeguard ecological health and maintain agricultural productivity.

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REFERENCES

Ajayi, A.O., Odeyemi, A.T., Akinjogunla, O.J., Adeyeye, A.B., Ayo-ajayi, I., 2024. Review of antibiotic-resistant bacteria and antibiotic resistance genes within the one health framework. Infection Ecology & Epidemiology 14, 2312953.

Ashton, D., Hilton, M., Thomas, K.V., 2004. Investigating the environmental transport of human pharmaceuticals to streams in the United

Kingdom. Science of The Total Environment 333, 167-184.
Bashaar, M., Thawani, V., Hassali, M.A., Saleem, F., 2017. Disposal practices of unused and expired pharmaceuticals among general public in Kabul. BMC Public Health 17, 45.
Beerling, D.J., Franks, P.J., 2010. The hidden cost of transpiration. Nature 464, 495-496.
Begum, M.M., Rivu, S.F., Hasan, M.M., Nova, T.T., Rahman, M.M., Alim, M.A., Uddin, M.S., Islam, A., Nurnahar, Tabassum, N., Moni, M.M., Roselin, R., Das, M., Begum, R., Rahman, M.S., 2021. Disposal Practices of Unused and Leftover Medicines in the Households of Dhaka Metropolis. Pharmacy 9.
Bigott, Y., Chowdhury, S.P., Pérez, S., Montemurro, N., Manasfi, R., Schröder, P., 2021. Effect of the pharmaceuticals diclofenac and lamotrigine on stress responses and stress gene expression in lettuce (Lactuca sativa) at environmentally relevant concentrations. Journal of Hazardous Materials 403, 123881.
Carter, L.J., Harris, E., Williams, M., Ryan, J.J., Kookana, R.S., Boxall, A.B.A., 2014. Fate and Uptake of Pharmaceuticals in Soil–Plant Systems. Journal of Agricultural and Food Chemistry 62, 816-825.
Copolovici, L., Pag, A., Kännaste, A., Bodescu, A., Tomescu, D., Copolovici, D., Soran, M.-L., Niinemets, Ü., 2017a. Disproportionate photosynthetic decline and inverse relationship between constitutive and induced volatile emissions upon feeding of *Quercus robur* leaves by large larvae of gypsy moth (*Lymantria dispar*). Environmental and Experimental Botany 138, 184-192.
Copolovici, L., Timis, D., Taschina, M., Copolovici, D., Cioca, G., Bungau, S., 2017b. Diclofenac Influence on Photosynthetic Parameters and Volatile Organic Compounds Emission from *Phaseolus vulgaris* L. Plants. Revista De Chimie 68, 2076-2078.
Dautermann, O., Lohr, M., 2017. A functional zeaxanthin epoxidase from red algae shedding light on the evolution of light-harvesting carotenoids and the xanthophyll cycle in photosynthetic eukaryotes. The Plant Journal 92, 879-891.
Duan, J., Liu, J., Huang, Z., 2024. Predicting the distribution pattern changes of dye plant habitats caused by climate change. Frontiers in Plant Science 15.
Garduño-Jiménez, A.-L., Carter, L.J., 2024. Insights into mode of action mediated responses following pharmaceutical uptake and accumulation in plants. Frontiers in Agronomy 5.
Gerke, J., 2022. The Central Role of Soil Organic Matter in Soil Fertility and Carbon Storage. Soil Systems 6, 33.
Granger, A.J., Nicoll, R.A., 2014. Expression mechanisms underlying long-term potentiation: a postsynaptic view, 10 years on. Philosophical Transactions of the Royal Society B: Biological Sciences 369, 20130136.
Hao, Q., Zhang, G., Zuo, X., He, Y., Zeng, H., 2022. Cia Zeaxanthin Biosynthesis, OsZEP and OsVDE Regulate Striped Leaves Occurring in Response to Deep Transplanting of Rice. International Journal of Molecular Sciences 23, 8340.
Ibrahim, M.H., Jaafar, H.Z.E., 2012. Impact of Elevated Carbon Dioxide on Primary, Secondary Metabolites and Antioxidant Responses of *Eleais guineensis Jacq.* (Oil Palm) Seedlings. Molecules 17, 5195-5211.
Jawahar Jothi, G., Sharma, D.K., Kovilpillai, B., Bhatia, A., Kumar, S., Antille, D.L., 2024. Interactive effects of elevated ozone and carbon dioxide on physiological traits of different Indian mustards. Plant Physiology Reports 29, 332-342.
Kayode-Afolayan, S.D., Ahuekwe, E.F., Nwinyi, O.C., 2022. Impacts of pharmaceutical effluents on aquatic ecosystems. Scientific African 17, e01288.
Kookana, R.S., Williams, M., Boxall, A.B.A., Larsson, D.G.J., Gaw, S., Choi, K., Yamamoto, H., Thatikonda, S., Zhu, Y.-G., Carriquiriborde, P., 2014. Potential ecological footprints of active pharmaceutical ingredients: an examination of risk factors in low-, middle- and high-income countries. Philosophical Transactions of the Royal Society B: Biological Sciences 369, 20130586.
Krupka, M., Piotrowicz-Cieślak, A.I., Michalczyk, D.J., 2022. Effects of antibiotics on the photosynthetic apparatus of plants. Journal of Plant Interactions 17, 96-104.
Lupitu, A., Moisa, C., Bortes, F., Peteleu, D., Dochia, M., Chambre, D., Ciutină, V., Copolovici, D.M., Copolovici, L., 2023. The Impact of Increased CO_2 and Drought Stress on the Secondary Metabolites of Cauliflower

(*Brassica oleracea* var. *botrytis*) and Cabbage (*Brassica oleracea* var. *capitata*), Plants.

Niinemets, Ü., Copolovici, L., Hüve, K., 2010. High within-canopy variation in isoprene emission potentials in temperate trees: implications for predicting canopy-scale isoprene fluxes. Journal of Geophysical Research - Biogeosciences 115, G04029.

Opriş, O., Copaciu, F., Soran, M.L., Ristoiu, D., Niinemets, Ü., Copolovici, L., 2013. Influence of nine antibiotics on key secondary metabolites and physiological characteristics in *Triticum aestivum*: leaf volatiles as a promising new tool to assess toxicity. Ecotoxicology and Environmental Safety 87, 70-79.

Park, Y.J., Park, S.-Y., Valan Arasu, M., Al-Dhabi, N.A., Ahn, H.-g., Kim, J.K., Park, S.U., 2017. Accumulation of Carotenoids and Metabolic Profiling in Different Cultivars of Tagetes Flowers. Molecules 22, 313.

Siddique, K.H.M., Bolan, N., Rehman, A., Farooq, M., 2024. Enhancing crop productivity for recarbonizing soil. Soil and Tillage Research 235, 105863.

Taschina, M., Copolovici, D.M., Bungau, S., Lupitu, A.I., Copolovici, L., Iovan, C., 2017. The influence of residual acetaminophen on *Phaseolus vulgaris* L. secondary metabolites. Farmacia 65, 709-713.

Taschina, M., Moisa, C., Lupitu, A., Copolovici, D.M., Copolovici, L., 2022. Influence of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) on Photosynthetic Parameters and Secondary Metabolites of Plants from *Fabaceae* Family. Applied Sciences-Basel 12.

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