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Decreased Soil Water Content Effects on the Toxicity of Triclosan to Oilseed Rape (Brassica napus L.)

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Abstract. Due to the rising amounts of antimicrobial agents in the environment and the lack of knowledge on their ecotoxicity, there is growing concern regarding their effects on the environment. One of the most widely used antibacterial compounds in both personal care and pharmaceutical products is triclosan (TCS), which is also a commonly detected emerging organic contaminant in the environment. Physiological or morphological endpoints of whole-organism analysis are typically used in reported studies of TCS toxicity to terrestrial plants. To identify underlying toxicity mechanisms, more in-depth investigations of TCS-induced effects at the biochemical plant level are required. Furthermore, climate change is an issue that is becoming more and more serious and might have a significant impact on life on the Earth. The influence of climate parameters on the ecotoxicity of antimicrobials, particularly TCS, is little understood. The main objective of this study was to evaluate drought effect on triclosan toxicity to oilseed rape (*Brassica napus* L.). Brassica napus were grown in TCS-contaminated soil (10-400 mg kg $^{-1}$) under different soil water contents (5% and 30% SWC). *B.napus* morphological (dry weight, length of the roots and shoots), biochemical indicators (the activity of enzymes), and the damage of oxidative stress (lipid peroxidation) were detected. Drought enhanced the negative effect of triclosan on the above-ground part of *B. napus* and led to oxidative stress.

Keywords: Triclosan, Climate change, Brassica napus

Introduction

There is growing concern about the environmental effects of antimicrobial chemicals because of their increasing levels in the environment, lack of information about their ecotoxicity on living organisms, and bacterial resistance. Triclosan (TCS), a broad spectrum antibacterial and antifungal agent, is extensively used in personal care products such as soaps, toothpastes as well as textiles, toys, implantable medical devices (Bedoux et al. 2012) and it is one of the most frequently detected emerging organic contaminant (EOCs) in the environment. Since the coronavirus (COVID-19) outbreak, TCS production and use rates have increased as a result of the rising demand for disinfection (Usman and Ho 2021). The excessive use and disposal of TCS containing disinfectants and antiseptics raises concerns about the negative effects on human health and the environment (Mukherjee et al. 2021). TCS has become one of the top ten most detected organic contaminants in the water (Brausch and Rand 2011). TCS is one of the most common pollutants in sewage sludge because it rapidly absorbs into the sludge (McClellan and Halden 2010). Direct sewage sludge application causes a large amount of TCS to be discharged into agricultural soils, presenting a high risk for entering the food chain via plants and soil organisms. Even though aquatic and terrestrial plants can accumulate many organic contaminants (Mathews et al. 2014), only several studies investigated ecotoxicological TCS effects on aquatic and terrestrial plants. A increasing amount of research also suggests that the fate, distribution, and toxicity of environmental toxins will be significantly impacted by climate change (Maulvault et al. 2016). According to a number of recent research, the toxicity of TCS to aquatic invertebrates changed as a result of changing climate factors (Maulvault et al. 2019; Pirone et al. 2019; Costa et al. 2020). However, up to date climate change effects on the TCS phytotoxicity were not studied at all.

2. Material and Methods

At Lithuania's Vytautas Magnus University, a pot experiment was conducted in a climate-controlled chambers. *B.Napus* were grown in a substrate consisting of loamy soil, perlite and fine sand (5:3:2, v/v). The TCS (Alfa Aesar GmbH & Co KG) was dissolved in acetone and applied to the soil at 6 different concentrations (in mg kg $^{-1}$ of soil): 10, 25, 50, 100, 200, 400. Each treatment was prepared in triplicates. The following environmental parameters were used to grow plants: a photoperiod of 14 hours, relative air humidity (RH) of 55–60%, and day/night air temperatures of 21/14±1 ºC. To maintain a consistent volumetric soil water content (SWC) of 30%, plants were watered using tap water. 35 days after sowing (DAS), the drought treatment period began, and it lasted 7 days. After watering ended, SWC steadily decreased and was maintained at ~ 5%. Three replicates of each treatment were tested.

50-100 mg tissue of *B. Napus* was homogenized with potassium phosphate buffer (pH 7, 50 mM), containing 2 mM dithiothreitol, 0.1 mM EDTA, and 1% of PVPP. The homogenates were centrifuged at a speed of 10 000 g for 15 minutes at 4 °C, and the supernatant was used for biochemical analysis. The Lowry et al. (1951) method was used to determine the soluble protein content of the tissues in the enzyme extract. The SOD activity was determined by the reduction of nitro blue tetrazolium chloride (NBT) at 560 nm (Dhindsa et al. 1981). The catalase (CAT) activity was assayed according to Aebi (1984) by monitoring decomposition of H_2O_2 at 240 nm (ε240 ¼ 0.0436 mM^{-1} cm^{-1}). Malondialdehyde (MDA) concentration was measured using a thiobarbituric acid-malondialdehyde (TBA-MDA) assay to determine lipid peroxidation (Murshed et al. 2008). All biochemical parameters were determined in 96-well microplates using SPECTROstar® Nano microplate reader (BMG LABTECH, Offenburg, Germany).

The statistical analyses were conducted using Statistica software. The effects of TCS concentration and water content (SWC) were evaluated using a Factorial (two-way) ANOVA. Pearson correlation coefficient was calculated between TCS concentration and evaluated parameters.

3. Results and discussion

Data on *B. napus* morphological parameters are presented in Fig. 1 (a, b, c, d). TCS concentration and water content interaction had significant effect on both shoot and root dry weight (ANOVA, F = 3.05 - 3.84, $p < 0.05$). Results showed that under both tested soil water contents (control and drought), TCS had negative impact for all tested *B. Napus* growth parameters. Strong negative correlation was detected (r= -0.978 – -0.853, $p > 0.05$) between TCS concentration and *B. napus* morphological parameters in both water regimes. However, TCS negative effect on *B. napus* aboveground part (shoot mass and length) was exacerbated by drought and lower shoot's dry biomass as well as length was observed at all tested TCS concentrations compared to the corresponding concentrations in the control.

Similarly to our results, it has been demonstrated that TCS inhibits plant emergence (Liu et al. 2009; Wang et al. 2015) and plant growth (EC_{50} 57–108 mg kg^{-1}) (Liu et al., 2009). Due to the lack of experiments, TCS negative impact mechanisms are not known yet. TCS, an antibacterial agent, could influence the microbial activity of the soil and damage soil qualities that are important for plant growth. According to research by Liu et al. (2009) and (Waller and Kookana), TCS impacted the nitrogen cycle and inhibited soil respiration. TCS was reported to inhibit algal growth, chlorophyll synthesis, and induce oxidative stress (Fekete-Kertész et al. 2018; Pan et al. 2018).

The action of many enzymatic antioxidants present in the plant tissues is necessary for the efficient scavenging of ROS produced during various environmental stressors (Sharma et al. 2012). In this work, two antioxidant enzymes (SOD, CAT) as well as lipid peroxidation (MDA concentration), a sign of oxidative damage, were evaluated as molecular biomarkers of oxidative stress. SOD is one of the most important components of antioxidant enzymes, which can catalyze O^{-2} radical into ordinary molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) (Hu et al. 2016). CAT perform important roles in the process of scavenging too many free radicals by antioxidant enzymes, which can break down H_2O_2 into H_2O and O_2 (Wu et al. 2012). Lipid membrane peroxide, or MDA, is a result of oxidative damage and can be used as an indirect measure of an organism's ROS level (Bacanlı et al. 2014; Song et al. 2019). Biochemical results of your experiment are presented in Fig. 3. TCS enhanced CAT activity in both water regimes and under control conditions strong correlation between CAT activity and TCS concentration was detected (r-0.79, $p < 0.05$). However, higher CAT activity at 100 - 400 mg TCS kg^{-1} concentrations was observed under control conditions in

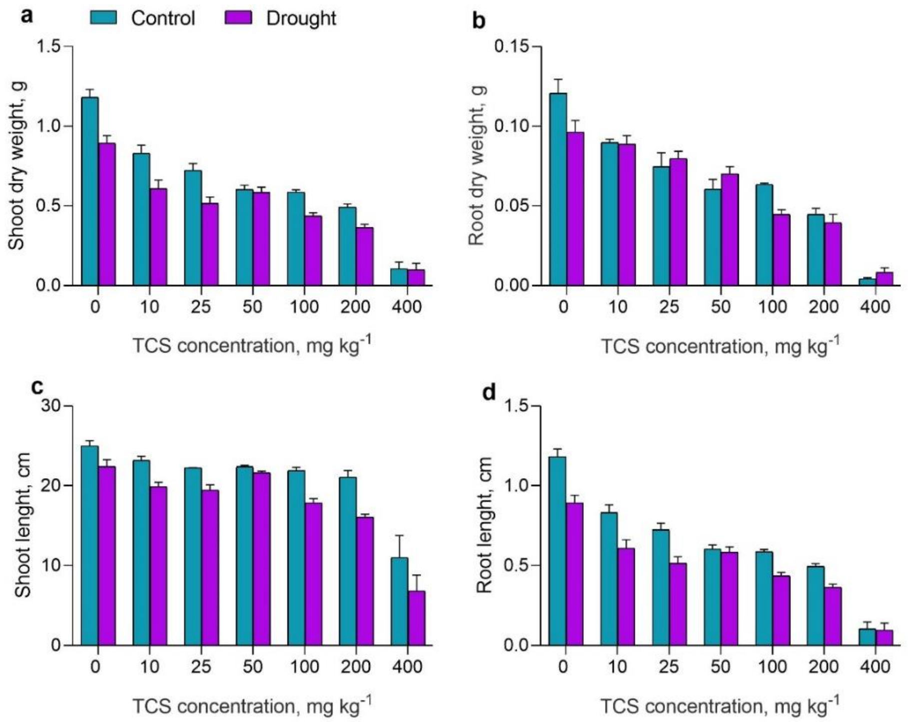

Fig 1. Effects of TCS to *B. napus* shoot (a, c) and root (b, d) dry weight and lenght under different soil water regimes

comparison with drought (Fig. 2 a). TCS enhanced SOD activity only under control conditions at 100 - 400 mg TCS kg $^{-1}$, whereas MDA levels did not increase (Fig. 2 c) and were at control (unexposed to TCS) levels. This might be due to enhanced SOD and CAT activity meaning that *B. napus* antioxidative system were able to detoxify free radicals. Drought enhanced *B. napus* lipid peroxidation and MDA levels increased significantly in comparison to control levels. Interestingly, under drought conditions SOD activity was not enhanced to cope with oxidative stress and slightly increased CAT activity were not able to detoxify free radicals. TCS is reported to include oxidative stress for various organisms (Zaltauskaite and Miskelyte 2018), however more studies is needed to understand underlying mechanisms.

4. Conclusion

Triclosan had a negative effect on *B. napus* growth in both water regimes. Under control conditions enzyme activity was enhanced thus lipid peroxidation did not occur. Drought enhanced the negative effect of triclosan on the above-ground part of *B. napus* and led to oxidative stress.

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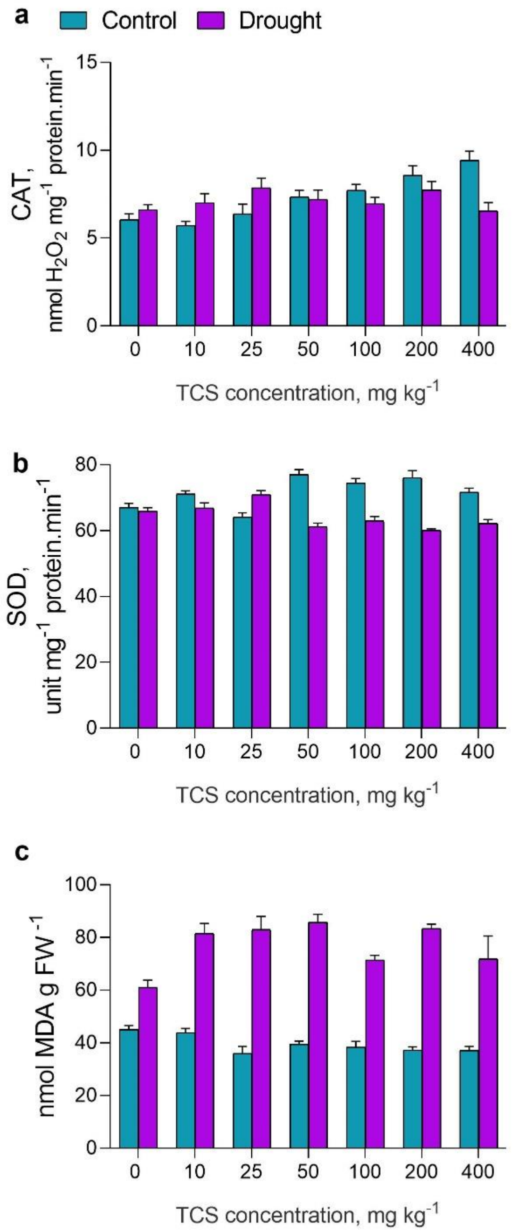

Fig 2. Effects of TCS exposure to CAT (a), SOD (b) activity and MDA levels (c) in *B. napus* leaf tissue under different soil water content.

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