Environmental Science and Pollution Research (2021) 28:14576–14585
https://doi.org/10.1007/s11356-020-11665-4

RESEARCH ARTICLE

Effects of the lichen *Peltigera canina* on *Cucurbita pepo* spp. *pepo* grown in soil contaminated by DDTs

Aysegul Akpinar[1] · Asuman Cansev[2] · Mehmet Isleyen[3]

Received: 16 April 2020 / Accepted: 12 November 2020 / Published online: 19 November 2020

Abstract

Lichens consisting of a symbiotic association of green algae or cyanobacteria and fungi are found in a variety of environmental conditions worldwide. Terricolous lichens, located in soils, affect the living and lifeless environment of the soil due to their effective secondary metabolite and enzymatic content. Terricolous lichens can increase the biological, chemical, and physical usefulness of soil. However, their effects in ensuring the bioavailability of contaminated soil are not known, especially on soil pollution caused by DDTs (*p,p'-DDE*, *p,p'-DDD*, *p,p'-DDT*). This research focuses on the effect of terricolous lichens on zucchini (*Cucurbita pepo* spp. *pepo*) grown in soil contaminated by DDTs, utilizing their secondary metabolite and enzymatic contents. Firstly, *Peltigera canina*, a terricolous lichen species, was added to soil contaminated by DDTs as powdered and intact thallus. After lichen addition to soil, zucchini was planted in. The oxidative stress and antioxidative enzyme activities of zucchini were measured. According to the results, *P. canina* treatments have a positive effect on the growth and development of zucchini, although oxidative stress was observed. Also, it was determined that powdered application had more effective results than intact thallus application.

Keywords Lichens · *Peltigera canina* · DDTs · Soil bioavailability · Oxidative stress · Antioxidative defense system

Introduction

Lichens are important part of terrestrial ecosystems. They occur in a large proportion of the ground-layer biomass of some forest, grassland, and tundra ecosystems (Asplund and Wardle 2017). Lichens consisting of a symbiotic association of green algae or cyanobacteria and fungi are a higher organism group in comparison to bacteria and fungi. They have their ability to tolerate difficulties in the most extreme environments (Beckett et al. 2008). They are on the vast parts of the Earth's land surface from low temperature, to high intensity of ultraviolet radiation and prolonged drought in the hot deserts (Nash 2008). Because they have improved a perfect adaptation evolutionally in order to survive in extreme conditions

Highlights
- *Peltigera canina* treatments (powdered and intact lichen thallus) have a positive effect on the growth and development of zucchini (*Cucurbita pepo* spp. *pepo*) grown in soils contaminated by DDTs.
- Especially powdered *Peltigera canina* can be added to the soil contaminated with DDTs as soil additive to induce development of zucchini.

Responsible Editor: Philippe Garrigues

✉ Aysegul Akpinar
agulugur@gmail.com

Asuman Cansev
auslu@uludag.edu.tr

Mehmet Isleyen
mehmet.isleyen@btu.edu.tr

[1] Vocational School of Higher Education, Bilecik Seyh Edebali University, 11230 Bilecik, Turkey

[2] Horticulture Department, Faculty of Agriculture, Bursa Uludag University, 16059 Bursa, Turkey

[3] Department of Environmental Engineering, Bursa Technical University, 16130 Bursa, Turkey

(Nardemir et al. 2015), lichens grow on various surfaces including trees, soil, and rocks, sometimes even on organisms like lichens and moss. Therefore, they take different names according to the type of substrate that they are located in. For example, the name of lichens that are located in soil is terricolous while the name of lichens that is on plants is epiphytes. Thus, several studies have been performed on the lichen effect on living organism or non-living area and/or the effects of them on lichens (Favero-Longo and Piervittori 2010). However, they are limited in scope, such as interaction of epiphytes with air pollution (Franc and Mayrhofer 1996; Dymytrova 2009) or plant tissues which is used as a substrate (Nash 2008; Koopmann et al. 2007) or competitive relationships between terricolous lichens and plants (Lawrey 2009). The effects of *Cladonia stellaris* on pine seed germination and growth of mycorrhizal fungi were observed by Sedia and Ehrenfeld (2003). It was determined by some authors the biodeterioration or bioprotection effects on lichen-plant interactions (Chen et al. 2000; Piervittori et al. 2009). In a previous study, we found that some terricolous lichens (*Cladonia rangiformis* Hoffm., *Peltigera neckerii* Hepp ex Müll. Arg., *Peltigera rufescens* (Weiss) Humb.) alter soil mineralization and microorganism content because of their strong secondary metabolites and enzymatic activities (Akpınar et al. 2009). Based on this, terricolous lichens can increase the biological, chemical, and physical usefulness of soil with its effective secondary metabolite and enzyme content. Also, it can be effective in ensuring the bioavailability of contaminated soil (Valencia-Islas et al. 2007).

Persistent organic pollutants (POPs) are still one of the important contaminants because of their high resistance to degradation, toxicity, and potential for bioaccumulation in ecosystem in the world (Wang et al. 2004; White 2009). The contaminants can be observed anywhere from the Arctic poles, where no agricultural practices exist, to all terrestrial areas due to their atmospheric movements (Kelly and Gobas 2001; Klánová et al. 2008). 2,2-bis(chlorophenyl)-1,1,1-trichloroethane (DDT), classified as POPs, is converted into 2,2-bis(chlorophenyl)-1,1-dichloroethane (DDD) and 2,2-bis(chlorophenyl)-1,1-dichloroethylene (DDE) metabolites by biotic or abiotic reactions in the soil (Foght et al. 2001). They can be still measured in decades due to their extreme accumulative and long-half-live traits in soil and biological organisms. Exposure to DDT (sum of DDT and its metabolites) residues causes different toxicity in different organisms, such as nausea, headache, tremors, and confusion in humans (Purnomo et al. 2011; Singh et al. 2016); inhibition on growth of crops like rice (*Oryza sativa*), barley (*Hordeum vulgare*), mung bean (*Vigna radiata*), pigeon pea (*Cajanus cajan*), and cotton (*Gossypium hirsutum*) (Mitra and Raghu 1989); and limitation of male development in animals because DDTs are estrogenic (Foght et al. 2001). Because DDTs are hydrophobic (log Kow > 3.5), they rapidly reduce the bioavailability of the soil by tightly binding to the organic substance in the soil or sediment and over time passing to the innermost structures of natural soils (Alexander 2000). In a study conducted by our research group (Isleyen et al. 2013), DDT residues were found in more than 90% of the soil samples taken from the intensive areas of agriculture in Karasu-Sakarya (Turkey). It has been reported that the total amount of DDT in the soils in the Karasu-Sakarya area is between 504 and 3557 ng/g. DDTs are still the main factor of soil pollution for many areas and were known to disrupt biological and physical structure of soil.

Certain *Cucurbita* species is well known to have significant potential of DDT accumulation and thus, they are often used in various phytoremediation approaches (Huelster et al. 1994; White et al. 2003, 2006; Mattina et al. 2006; Parrish et al. 2006; White 2009). Especially, DDE accumulation of zucchini *Cucurbita pepo* spp. *pepo* (cv. Raven) has been stated to 5-30-fold increase (White et al. 2007). Another study in this matter states that zucchini uptakes the same organic pollutant from soil and is collected in different parts such as root, stem, petiole, and leaf blade (Gent et al. 2007). In the studies, zucchini has repeatedly been shown to extract high levels of weathered *p,p′*-DDE from soil. It is clear that the zucchini is a good DDT accumulator rather than contributor to bioavailability of DDT-contaminated soil. Similarly, there are a few studies as well about DDT accumulation in lichens (Kelly and Gobas 2001). Lichens are known to play a leading role in ecological succession formation and to have effective secondary metabolite contents. However, the effects of lichens have not been investigated on soil ecosystem and soil pollution caused by DDTs yet.

For this purpose, in our experiment, we determine the effect of terricolous lichens on zucchini *Cucurbita pepo* spp. *pepo* grown in soil contaminated with DDTs, utilizing their secondary metabolite and enzymatic contents. Since the separation of the lichen secondary metabolite will not yield effective results as shown in the studies of Kytöviita and Stark (2009), lichen thallus was handled preserving its existing structure, even in powder form. *Peltigera canina*, one of the terricolous lichen species, was chosen and added to DDT-contaminated soil as powdered and intact thallus. The effects of *P. canina* on DDT-contaminated soil were stated by monitoring zucchini *Cucurbita pepo* spp. *pepo* (cv. Raven). Zucchini was chosen because it is accustomed to the DDT-contaminated soil. DDTs cause cellular stress by disrupting metabolic pathways or membrane integrity in plants and increase reactive oxygen species (ROS) production that damage membrane lipids, enzyme activation and DNA, and etc. (Mitton et al. 2014). Therefore, the enzymatic (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX)) and non-enzymatic (glutathione) analyses which belong to the antioxidative defense system of zucchini were investigated as a priority. These analyses were supplemented

by lipid peroxidation, chlorophyll contents, and etc. At the same time, chlorophyll content in lichens was measured because of being one of the important ecological parameters used for the assessment of degree of various environmental stress such as heavy metals and air pollution (Chettri et al. 1998; Garty et al. 2000; Dzubaj et al. 2008).

Material and methods

Collection of lichen samples

A total of 22 lichen species belonging to the genus *Peltigera* are found in 198 locations across Turkey (Turk et al. 2015). Eleven of these locations belong to Bursa and its surroundings. In this study, the lichens belonging to the species *Peltigera canina*, which is the most abundant in these locations, were collected. Lichen thalli which were examined in this study were collected from one location, in March 2018 in Bursa, altitude: 484 m, N: 39° 38′ 36″, E: 29° 01′ 08″. Ten to twelve lichen thalli of similar size were used. The lichen species collected from nature were separated from their substrates and cleaned in the laboratory. While some of it was powdered, the integrity of the other lichen thallus was left intact (Fig. 1 a).

Supply of *p,p′*-DDE-contaminated soils

Soil samples used in the experiments to be designed within the scope of this study were taken from 0 to 30 cm depth of area contaminated with DDTs in Karasu-Sakarya/Turkey. They were passed through 2-mm sieves, and stone, plant residues, and other impurities were removed. Afterwards they were dried in room conditions and stored in 5-L volume clean glass jars with the lid closed in the laboratory. In this study, the amount of DDTs in each application was set to on average 400 ng/g dry soil. This value was determined by considering the values of past DDT usage (White et al. 2003, 2007; White 2009).

Cultivation of zucchini plants

In the current study, zucchini *Cucurbita pepo* spp. *pepo* (cv. Raven) seeds purchased from Johnny's Selected Seeds (Albion, ME) were used. Seeds were allowed to germinate in dark conditions in an incubator set at 20 °C in a moist filter paper. Estimated germination time is between 8 and 10 days. The germinated seeds were transferred to viols and grown with Hoagland solution in a growth chamber (day/night temperature 25 °C/15 °C, 16 h photoperiod, relative humidity 60%). The seedlings were grown in these conditions for 8 weeks (Fig. 1 b).

Experimental design

Eight-week-old plants were subdivided into three treatments/cases. The treatments are expressed below and schematically presented in Fig. 1 c:

1. Soil with *p,p′*-DDE (control),
2. Soil with added powdered lichen and mixed with *p,p′*-DDE,
3. Soil mixed with *p,p′*-DDE and planted intact lichen thallus.

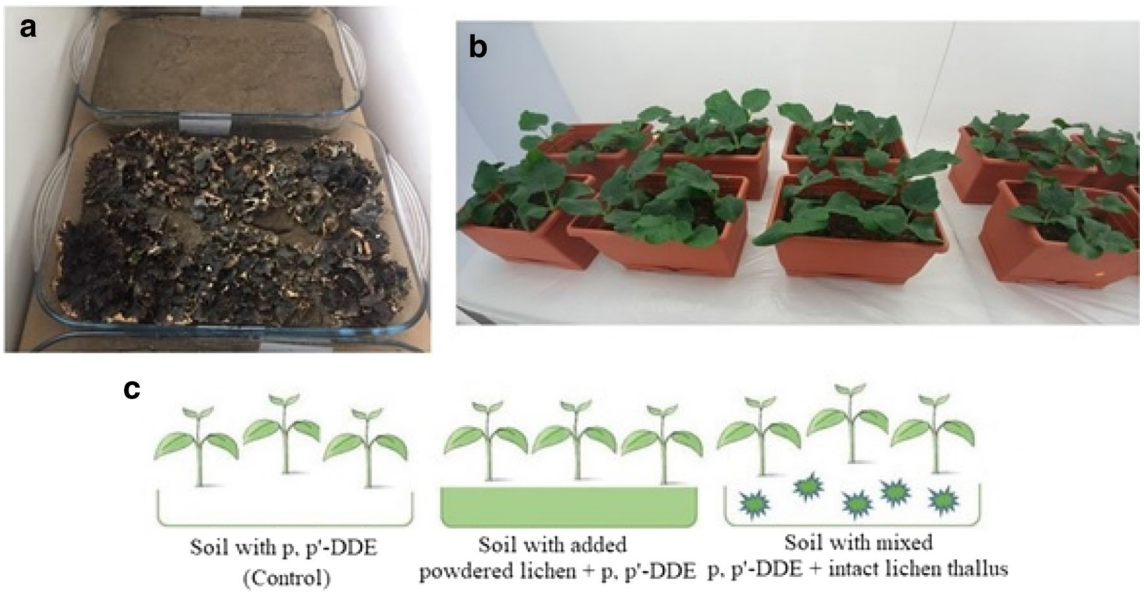

Fig. 1 Experimental design of the study. **a** View of *Peltigera canina* added as powdered and intact thallus to soil contaminated by DDTs. **b** The zucchini (*Cucurbita pepo* spp. *pepo*) seedlings grown in controlled conditions for 8 weeks. **c** Schematic representation

While powdered lichen (*Peltigera canina*) was added to the 2nd case, intact lichen thallus of *Peltigera canina* was added to the 3rd case. Zucchini seedlings were cultivated in all the soil combinations during 12 weeks. Five plants per case with three replications were applied on the experiment. At the end of 12 weeks of experiment, plant samples were washed thoroughly with de-ionized water and separated into root and leaf portions. Fresh plant materials were used for the determination of chlorophyll content; the rest of the samples were stored at − 70 °C after freezing in liquid nitrogen.

Chlorophyll pigment analysis

The chlorophyll content (Chl *a*, Chl *b*, and total Chl) for zucchini was determined spectrophotometrically according to the Arnon (1949) method. Fresh leaves (0.05 g fresh weight; FW) were homogenized with 10 mL of 80% acetone, and then filtered. The absorbance of samples was measured at 645 and 663 nm (Novaspec II, LKB Biochrom).

In determination of the chlorophyll content for lichen (*Peltigera canina*), 20 mg lichen material using 5-mL pure DMSO (dimethyl sulphoxide (for synthesis) 99% purity, Merck) was used. Then, tubes containing DMSO and lichen material were incubated at 65 °C for 40 min in the dark and then allowed to cool to room temperature. The extracts were filtered with a Whatman no. 3 filter paper. The spectrophotometer was calibrated at 750 nm with DMSO. Absorbance of the extracts was read at 665 and 648 nm for calculating Chl *a*, Chl *b*, and total chlorophyll (Chl $a = 14.85\ A^{665} - 5.14\ A^{648}$, Chl $b = 25.48\ A^{648} - 7.36\ A^{665}$, Chl $a + b = 7.49\ A^{665} + 20.34\ A^{648}$). Chlorophyll extractions were done according to Barnes et al. (1992).

Lipid peroxidation

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) determined by thiobarbituric acid (TBA) reaction, and it was expressed as nmol/g fresh weight. MDA content was determined described by Heath and Packer (1968) with some modifications. A total of 0.1 g FW of leaf sample was homogenized in 0.5 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000*g* for 10 min. Then, the reaction mixture containing 0.5 mL of the supernatant and 1.5 mL of the mixture of 20% TCA and 0.5% TBA was added into new tubes. After incubation at 95 °C for 30 min, the tubes were cooled with ice and centrifuged (15,000*g*, 4 °C for 5 min). The absorbance of the mixture was read at 532 and 600 nm (Novaspec II, LKB Biochrom). The MDA concentration was calculated using the extinction coefficient ($\mathcal{E}$) of 155 $mM^{-1}\ cm^{-1}$.

Antioxidant enzyme activity

Plant material (1 g) was homogenized with 3 mL of buffer solution containing 50 mM Na-phosphate buffer (pH 7.8), 1 mM EDTA, and 2% (w/v) polyvinylpolypyrrolidone (PVP) in an ice bath. Homogenized materials were centrifuged at 14,000*g* for 40 min at 4 °C. The supernatants were transferred into Eppendorf tubes for the determination of enzymatic activities. This supernatant was used to determine the superoxide dismutase (SOD) and catalase (CAT) activity. For the ascorbate peroxidase (APX) activity assay, 2 mM ascorbate was added to the extraction buffer solution described above.

Superoxide dismutase assay

The SOD (EC 1.15.1.1) activity was determined by the method of Beauchamp and Fridovich (1971). This method is based on the inhibition of the nitroblue tetrazolium at 560 nm. The total mixture (3 mL) in the SOD activity assay contained 0.5 mL of the enzyme extract, 2.5 mL of an ice cold buffer containing 20 mM sodium phosphate buffer (pH 7.5), 0.1 mM EDTA, 10 mM methionine, 0.1 mM *p*-nitroblue tetrazolium (NBT), and 5 μM riboflavin. Then, control and sample test tubes were placed under 300 μmol $m^{-2}\ s^{-1}$ fluorescent light for 15 min and the reaction was stopped by switching off the light. The absorbance of the solution was measured at 560 nm (Novaspec II, LKB Biochrom) generated by the inhibition of the reduction of NBT. The SOD assay kit (SOD S7446, Sigma-Aldrich, USA) was used in the preparation of standards. After the calculation of % inhibition, the enzyme activity was determined according to the linear equation which is obtained from the curve and expressed as U/mg protein.

Catalase assay

The CAT (EC 1.11.1.6) activity was assayed as described by Lester et al. (2004) with some modifications. The reaction was initiated by adding 0.1 mL of the enzyme extract to the assay mixture containing 20 mM sodium phosphate buffer (pH 6.8) and 15 mM H_2O_2. The change in absorbance was measured at 240 nm for 3 min (Shimadzu UV-2100). The activity of enzyme was expressed as units per mg protein.

Ascorbate peroxidase assay

The APX (EC 1.11.1.11) activity was determined spectrophotometrically according to the method described by Lester et al. (2004). This enzyme activity was stated from the decrease in absorbance at 290 nm as ascorbate was oxidized. The reaction mixture comprised 50 mM potassium phosphate (pH 6.6), 0.25 mM ascorbate and 1 mM H_2O_2 (3% H_2O_2), and 1 mL of the enzyme extract. The change in absorbance was

measured at 290 nm for 3 min (Shimadzu UV-2100). The activity of enzyme was expressed as U/mg protein.

Non-enzymatic antioxidant: glutathione assay

Glutathione (GSH) determination was performed by the method reported by Ellman (1959). In this analysis, while GSH is oxidized by DTNB, disulfide bonds formed by oxidized glutathione and other soluble thiol compounds in the medium are converted to GSH by reduction of NADPH in the presence of glutathione reductase enzyme. The amount of 2-nitro-5-thiobenzoic acid which is color yellow is measured at 412 nm. For this, frozen leaf sample (0.2 g) was homogenized in ice cold 5% (w/v) trichloroacetic acid and centrifuged at 15,000*g* for 15 min. Then, 2.6 mL phosphate buffer (pH 7.7) and 0.2 mL DTNB (5,5'-dithiobis (2-nitrobenzoic acid) (2.51 mg mL^{-1})) were added to 0.2 mL supernatant. After 5 min at 30 °C, the absorbance was determined spectrophotometrically at 412 nm. Glutathione (GSH) content was calculated based on a standard curve (Ellman 1959).

Statistical analysis

In each treatment containing five plants, all analyses were performed with three replicates ($n = 3$). All the statistical tests were performed at a significance level of 0.05 using SPSS Statistics (version 22.0, IBM Corp., Chicago, IL). The data were statistically analyzed by one-way analysis of variance (ANOVA), with the means compared by Tukey's test.

Results and discussion

Chlorophyll contents (Chl *a*, Chl *b*, and total Chl) in zucchini grown in soil contaminated by DDTs mixed with *Peltigera canina* (powdered and intact thallus) and without *P. canina* (control) are shown in Fig. 2 a. According to this, chlorophyll content of zucchini growing in DDT-contaminated soil treated with *Peltigera canina* increased compared with the control plants ($p < 0.05$). This increase is associated with Chl *a* content. There was no statistically significant change in Chl *b* content of zucchini, thus leading to an increase in the Chl *a*/*b* ratio (Fig. 2 b). This indicates that Chl *a* has the leading role in DDT pollution than Chl *b*. It is seen in several studies that Chl *a* is more sensitive to various pollution factors such as DDTs and PAHs (Lawler and Rodgers 1967; Chung et al. 2007; Mishev et al. 2009). In the present study, it was observed that *Peltigera canina* eliminated the negative effect of DDTs on Chl *a* of zucchini. In addition, Chl *a*/*b* ratio can serve to determine the physiological state of plant (Bačkor et al. 2003). According to our results, the presence of *Peltigera canina* in DDT-contaminated soil was positively affected by the physiological status of zucchini *Cucurbita pepo* spp. *pepo* (cv. Raven). In particular, the effect of intact lichen thallus is undeniable ($p < 0.05$, Fig. 2 b).

When we look at the chlorophyll content in the intact lichen thallus of *Peltigera canina*, an increase in the total chlorophyll content was detected in our results (Fig. 3). Changes in chlorophyll contents of *Peltigera canina* were again due to Chl *a* content. No statistically significant difference in Chl *b* contents of *Peltigera canina* was observed. Results which belong to Chl *a*/*b* ratio showed that DDT pollution has no photodestructive effect on *Peltigera canina* before and after application ($p < 0.05$). Chl *a*/*b* ratio in healthy intact lichens is generally considered to be 2-4 (Chettri et al. 1998; Bačkor et al. 2003). Our results are between (before application) and above (after application) these values (Fig. 3). From our results, we understand that the DDT pollution does not adversely affect the photosynthetic mechanism of *Peltigera canina*.

Lipid peroxidation is a good biomarker to determine the state of membrane lipids of oxidative stress, and estimated by the MDA level in this study (Fig. 4). *Peltigera canina* (intact thallus and powdered) treatments increased statistically MDA contents in roots and leaves of zucchini growing in DDT-contaminated soil ($p < 0.05$). The increase in roots was more than the leaves. Because, in particular, intact lichen thallus covered the DDT-contaminated soil like a blanket, roots of the zucchini plants were more affected. Bioactive components contained in *Peltigera canina* may have changed the rhizosphere of zucchini. This situation caused oxidative stress in roots of zucchini, leading to the formation of ROS. Against oxidative stress caused by free radicals (Rodriguez-Serrano et al. 2006; Wang et al. 2010), the antioxidative defense system of plants, which are subject to phytoremediation such as zucchini, needs to be investigated in detail (Shaw et al. 2004; Kim et al. 2008).

Plants have some enzymes and compounds to avoid oxidative damage caused by ROS. These enzymes and compounds form the antioxidative defense system in plants. This system is regulated by both enzymatic and non-enzymatic mechanisms. While non-enzymatic antioxidative system contains small molecular weight antioxidants such as glutathione, cysteine, hydroxyiquinone, ascorbate (vitamin C), α-tocopherol, carotenoid pigments, and alkaloids (Michalak 2006), superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR) (Shaw et al. 2004) take place in the enzymatic system. They play an important role in plant survival and adaptation to the environment under various stress conditions (Shao et al. 2008; del Rio 2015). In this study, tissue-specific responses (roots, stems, and leaves) of the antioxidative defense system in zucchini were investigated in detail and are shown in Fig. 5. SOD activity, which is the first step of enzymatic antioxidative defense system, increased in roots and leaves of zucchini grown in soil contaminated by

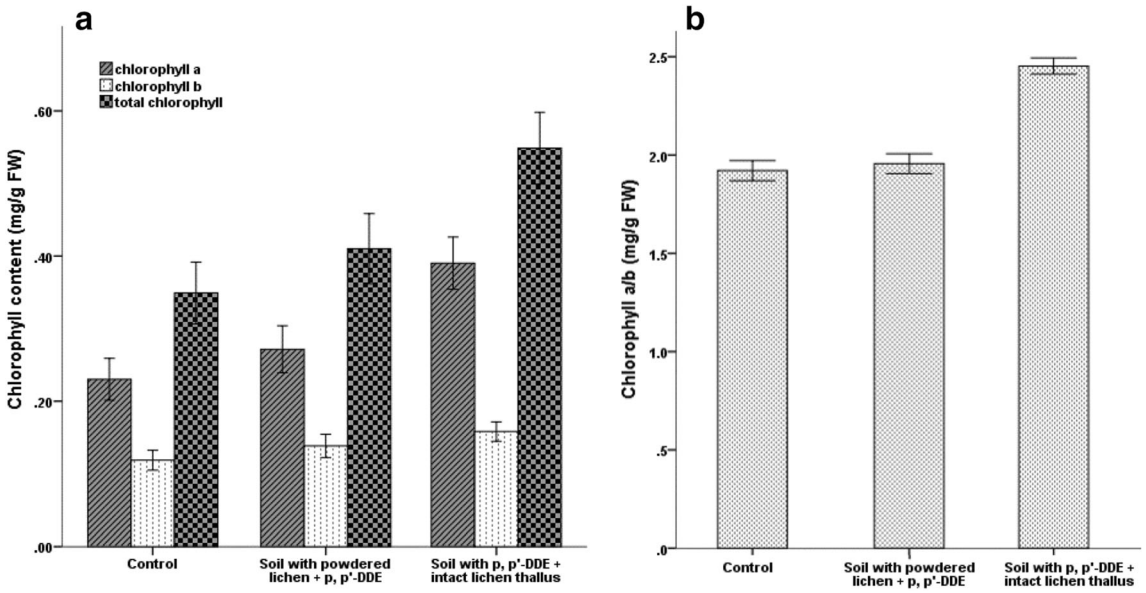

Fig. 2 **a** Chlorophyll content (chlorophyll *a*, *b*, and total chlorophyll). **b** Chlorophyll degradation rate (*a*/*b*) in leaves of zucchini grown in contaminated soil by DDTs mixed with *P. canina* (powdered and intact thallus) and without *P. canina* (control) (mg/g FW). Data points represent means and standard errors ($n = 3$)

DDTs mixed with *Peltigera canina* (both powdered and intact thallus, Fig. 5 a). But there was no statistically significant increase in the stems of zucchini ($p < 0.05$). It is seen that the stem of zucchini acts only as a transport vehicle from roots to leaves. Increased SOD activity was observed due to oxidative stress in the roots of zucchini due to changes in the rhizosphere caused by bioactive components of *Peltigera canina*.

ROS production in plants is generated with basic metabolic activities in various subcellular sites such as mitochondrial

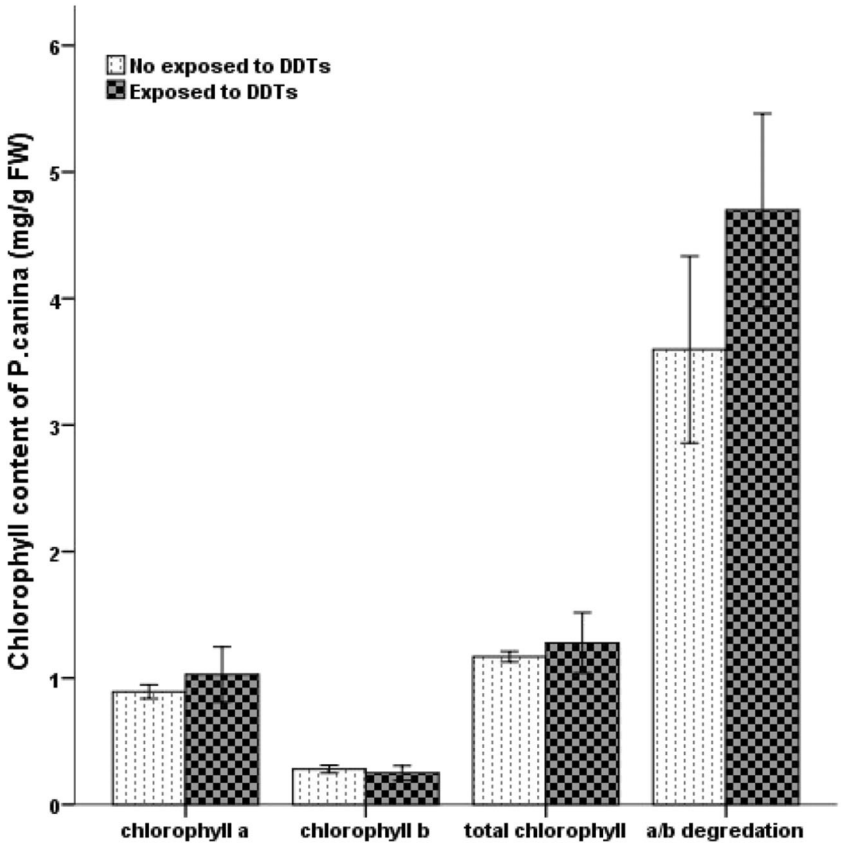

Fig. 3 Chlorophyll content (chlorophyll *a*, *b*, and total chlorophyll) and chlorophyll degradation ratio (*a*/*b*) in intact *P. canina* thallus (mg/g FW). Data points represent means and standard errors ($n = 3$)

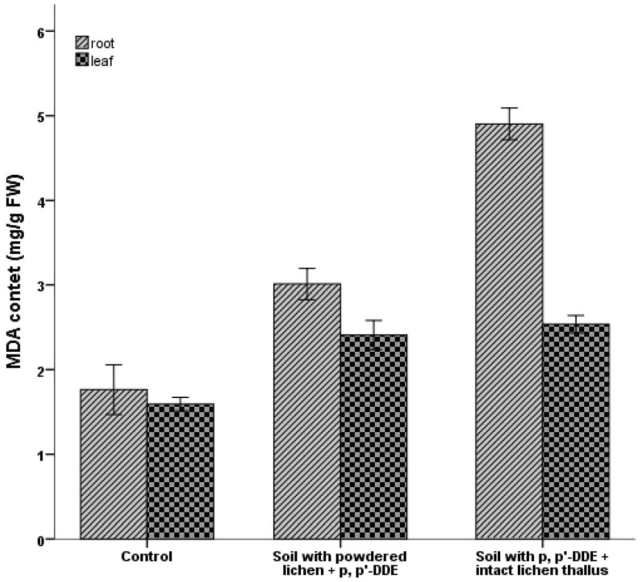

Fig. 4 Malondialdehyde (MDA) contents in roots and leaves of zucchini grown in contaminated soil by DDTs mixed with *P. canina* (powdered and intact thallus) and without *P. canina* (control) (mg/g FW). Data points represent means and standard errors ($n = 3$)

respiration, photosynthesis in chloroplasts, peroxisome-localized photorespiratory reactions, and apoplastic actions (Mhamdi and Van Breusegem 2018). Our results indicate that the pathway of ROS formation causing oxidative stress is thought to be originated by mitochondrial ETS, peroxisomal or apoplastic actions. If photosynthetic ETS-induced ROS formation had occurred, this would have caused structural damage and decreased chlorophyll content. However, we would have seen it during the evaluation of the photosynthetic processes of zucchini. In this study, it was determined that both lichen treatments (powdered and intact thallus) increased DDT accumulation in zucchini plants (Our article is under review). Parallel to this increase, ROS production in the leaves of zucchini has also been observed. SOD activity in the leaves of zucchini increased to prevent the damage caused by ROS (Fig. 5 a).

SOD activity catalyzed the dismutation of O_2^- to hydrogen peroxide (H_2O_2) to decrease the level of oxygen free radicals in cell. However, the generated H_2O_2 is also a free radical and needs to be eliminated. In this case, CAT and APX enzymes work to break up H_2O_2. Figure 5 b shows the CAT activity in roots, stems, and leaves of zucchini grown in soil contaminated by DDTs mixed with *P. canina* (powdered and intact thallus) and without (control). CAT activity was increased in the roots of zucchini in both forms (powdered and intact thallus) of *Peltigera canina* application ($p < 0.05$). When we look at the stems of zucchini, statistically significant results are seen only in the intact thallus application of *P. canina* ($p < 0.05$). Although the powdered application of *Peltigera canina* evoked a statistically significant increase in CAT activity of leaves of zucchini, 4-fold increase in intact thallus application was noticed (Fig. 5 b). This situation indicates that intact lichen thallus causes higher H_20_2 concentrations and ROS formation via peroxisomes in leaves of zucchini. Peroxisomes are effective in maintaining metabolic activities in the plants and CAT is a peroxisomal enzyme (Mhamdi et al. 2010). These results are connected with increased DDT accumulation in leaves of zucchini grown in soil contaminated by DDTs mixed with intact lichen thallus (under review). APX activity also was shown in Fig. 5 c. According to these results, it has given different results in the plant parts of zucchini. For example, APX activity increased in leaves of zucchini in both *Peltigera canina* treatments (powdered and intact thallus) compared to control. In zucchini's root and stem parts, while the application of powdered lichen caused decreases of APX activity, the intact thallus application induced increase in its activity ($p < 0.05$). In literature, it has been stated that activity of these enzymes shows difference in different parts of plants (Qiu et al.

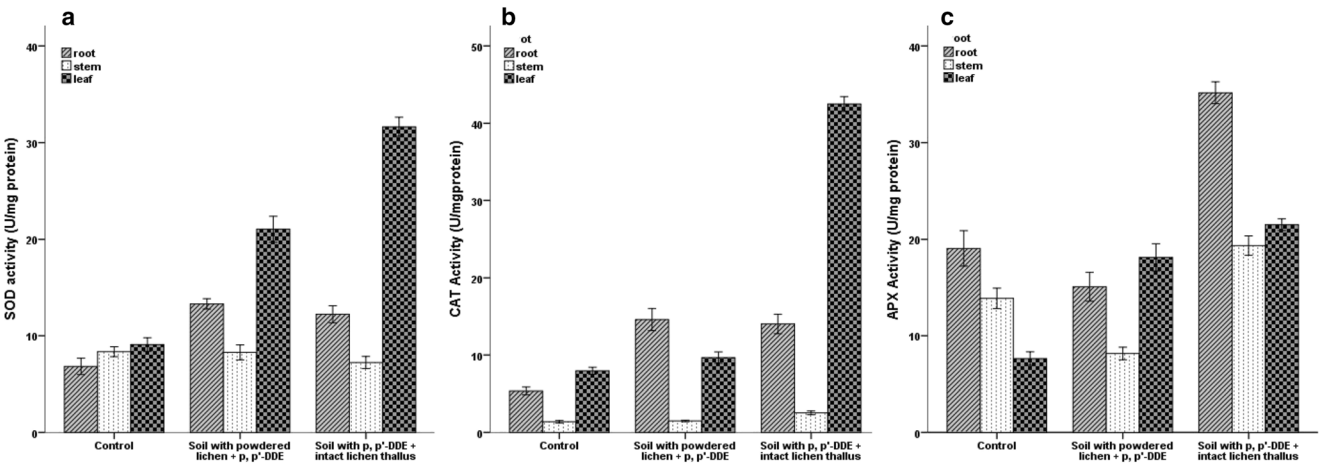

Fig. 5 Tissue-specific responses (roots, stems, and leaves) of enzymatic antioxidative defense system in zucchini *Cucurbita pepo* spp. *pepo*. **a** Superoxide dismutase (SOD) activity, **b** catalase (CAT) activity, **c** ascorbate peroxidase (APX) activity in roots, stems, and leaves of zucchini grown in contaminated soil by DDTs mixed with *P. canina* (powdered and intact thallus) and without *P. canina* (control) (U/mg protein). Data points represent means and standard errors ($n = 3$)

2008). In addition, APX in the elimination of H_2O_2 has higher affinity compared with CAT (Cansev et al. 2011). Similarly, APX activity was more effective in the dismutation of hydrogen peroxide for zucchini.

At the same time, APX is an important enzyme in ascorbate-glutathione cycle (Halliwell-Asada pathway) and disintegrates H_2O_2 by utilizing ascorbate as the reducing agent. Apart from the role of ascorbate and glutathione in Halliwell-Asada cycle, these compounds can act independently (Ozdener and Aydin 2010). Our results show that APX activity is not correlated to endogenous levels of the antioxidant metabolite glutathione (GSH) content in zucchini ($p < 0.05$). The GSH content is shown in Fig. 6. According to this result, GSH content decreased with *Peltigera canina* application in the root and leaf parts of zucchini. GSH has often been considered to play an important role in plant defense system against oxidative stress. When exposed to various stresses, it is shown that the levels of GSH change in plants (Noctor et al. 2002). In the present study, the decrease in GSH activity may have been caused by unproduced metabolic energy (NADPH) due to mitochondrial-induced ROS production in zucchini, because GSH need NADPH for its metabolic activity. There was no statistically significant change detected in the stems of zucchini (Fig. 6, $p < 0.05$).

Conclusions

Peltigera canina treatments (powdered and intact thallus) increased growth and photosynthetic pigments of zucchini grown in soil contaminated by DDTs. But it is also clear that these treatments cause oxidative stress in zucchini. Increasing antioxidant defense system enzymes (SOD, CAT, APX) is a protective mechanism in dealing with oxidative stress. On the other hand, the decrease in glutathione content suggests that mitochondrial-induced ROS occurs. Our study also made important contributions to the understanding of the physiology and defense system of zucchini grown in soil contaminated with DDTs.

Our results formed that terricolous lichens which have effective secondary metabolite and enzymatic contents may be effective in increasing the bioavailability of DDT-contaminated soils. It may be that especially powdered *Peltigera canina* added to soil contaminated with DDTs as soil additive will induce development of zucchini and have an effect on the phytoremediation potential of zucchini. Research about interactions of two different organisms are very complex and require multidisciplinary studies. In the future, investigation of other terricolous lichens will further unravel the additive roles in this process.

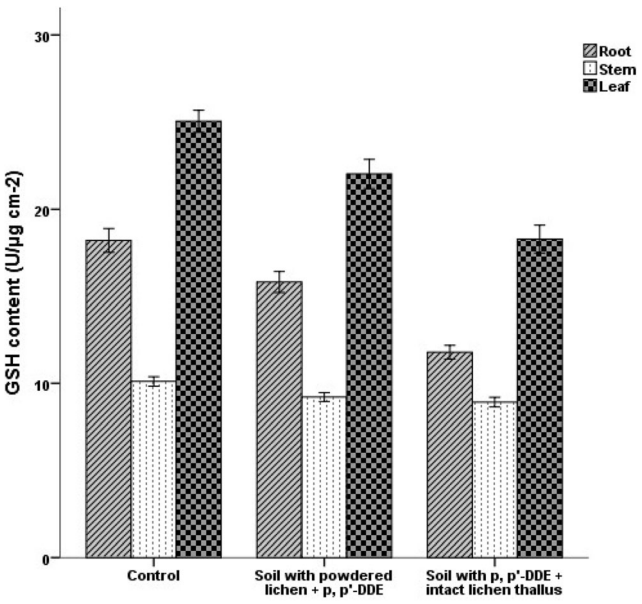

Fig. 6 Glutathione (GSH) content in roots, stems, and leaves of zucchini grown in contaminated soil by DDTs mixed with *P. canina* (powdered and intact thallus) and without *P. canina* (control) (U/µg cm^{-2}). Data points represent means and standard errors ($n = 3$

Authors' contributions Aysegul Akpinar and Mehmet Isleyen designed the study; Aysegul Akpinar carried out the analyses; Aysegul Akpinar and Asuman Cansev wrote the paper. All authors read and approved the final manuscript.

Funding This work was supported by the Scientific and Technological Research Council of Turkey (TUBITAK), Grant No: 117Y363.

Data availability The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval and consent to participate Not applicable.

Consent to publish Not applicable.

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