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Research Article

Effect of Phosmet Toxicity on Some Physiological Traits in Duckweed (*Lemna gibba* L.)

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ABSTRACT

Pesticides, one of the chemicals that adversely affect the environment and human health, can have effects on non-target organisms due to their chemical properties and wide range of use. Therefore, in this study, the toxic effects of phosmet insecticides on *Lemna gibba*, an aquatic macrophyte, were determined as a non-target organism. The study was carried out in a climate cabinet under controlled conditions. It was determined that the photosynthetic pigments and total carbohydrate content of the macrophyte decreased with increasing phosmet concentration. Similarly, decrease in total phenolic contents were found. A significant and positive correlation between non-protein sulfhydryl groups (NP-SH) and H_2O_2 contents may indicate their role in antioxidant defense mechanism. Besides, increases in malondialdehyde (MDA) and H_2O_2 contents showed that phosmet toxicity caused oxidative stress in *L. gibba* tissues.

Keywords: *Lemna gibba*, phosmet, toxicity, oxidative stress.

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INTRODUCTION

Pesticides are chemical substances that are used to control pests. Their persistent nature, poisonous qualities, bioaccumulation, lipophilicity, and detrimental effects on the environment and human health make them extremely concerning. Pesticide residues contaminate the land and water, build up in plants, make their way up the food chain, and finally end up in human diets and water sources. As a result, they negatively impact non-target species (Barcelo and Hennion, 1997; Taylor et al., 2003).

Different standards have been used to classify pesticides. Mechanism of action and/or mechanism of entrance, method of controlling or killing the target organism, chemical composition, and pesticide properties are the most often used classification criteria. The main classification of insecticides, herbicides, and fungicides is based on the target organism group (Drum, 1980; Hassaan and El Nemr, 2020).

Pesticide poisoning has a negative impact on plants' growth and development because of its impacts on metabolism. According to Sharma et al. (2019), their toxicity results in a drop in photosynthetic pigments and a decrease in photosynthetic efficiency. Reactive oxygen species are created when oxidative stress is triggered by pesticide damage. Plants have both enzymatic and non-enzymatic antioxidant defense systems that work to lessen the harmful consequences of oxidative stress (D'Souza, 2017; Sharma et al., 2019).

Phosmet, non-systemic and broad-spectrum organophosphate, is an anti-cholinesterase chemical used to control aphids, suckers, moths and sucker on plants and animals (FAO, 2019). The runoff and spray drift are main routes for it to enter water bodies and the reported maximum phosmet concentrations in ground- and surface water were 0.20 and 0.63 µg/L (United States Environmental Protection Agency (US EPA), 2010).

Limited data on other organophosphorus insecticides indicate that they may have the potential for toxic effects on aquatic plants. However, these effects vary depending on the chemical substance and species tested. On the other hand, it is not known whether phosmet will have similar impacts on non-target plants (United States Environmental Protection Agency (US EPA), 2010).

The present study was performed to evaluate the toxic effects of phosmet on (i) photosynthetic pigments (*chlorophyll-a*, *chlorophyll-b* and carotenoid), (ii) total carbohydrates, (iii) phenolic compounds and potential to cause oxidative stress by occupying biomarkers of non-protein sulfhydryl groups (NP-SH), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in an aquatic free-floating macrophyte, *Lemna gibba*. *L. gibba* was chosen as experimental model organism because of its advantages of ease of culture, quick development, and tiny size (Park et al., 2021).

MATERIALS AND METHODS

L. gibba was collected from water bodies in Gaziantep province (Türkiye). The macrophytes brought to the laboratory were acclimatized in a controlled climate cabinet (light level 120 µE $m^2.s^{-1}$, temperature 23±1 °C, Snijders Scientific, Netherlands) for two weeks in containers containing 10% nutrient solution (Dogan et al., 2021). After the macrophytes were acclimated to the experimental conditions, they were randomly divided into four groups. Stock phosmet solution was prepared by dissolving in acetone. The final concentration of acetone in the assay was 0.01% (v/v). Group I containing pesticide-free water with 0.01% acetone was used as control. Group II, III and IV were exposed to 0, 0,1, 10 and 100 ppm phosmet for 96 hours. 96 hours test duration was performed due to rapid hydrolysis of phosmet in aqueous environment with with half-lives of 13 days at pH 4.5, 12 hours at pH 7 and 4 hours at pH 8.3 (Tomlin, 2004). The applications were carried out in glass beakers containing 10% nutrient solution at concentrations of 200 mL. Deionized water used for preparing the solutions. At the end of treatment, fresh *L. gibba* samples were harvested and immediately stored at -80 °C until analysis.

To determine photosynthetic pigment contents, duckweed fronds were homogenized in 80% acetone. Following readings in a UV/VIS spectrophotometer, the contents of photosynthetic pigment (*chlorophyll-a, chlorophyll-b*, and carotenoid) were computed in accordance with Lichtenthaler and Wellburn (1985). The anthron method was used to determine the total soluble carbohydrate content of the macrophyte (Plummer, 1998). Glucose was used as standard in carbohydrate calculations. Non-protein sulfhydryl groups (NP-SH) were made according to Ellman's method (Ellman, 1959). Reduced glutathione (GSH) standard was used in NP-SH calculations. Total phenolic content was determined using Folin-Ciocalteu reagent (Ratkevicius et al., 2003). The curve of the gallic acid standard was used for total phenolic calculations. The lipid peroxidation level was determined by detecting the amount of malondialdehyde (MDA) using the method proposed by Zhou (2001). The H_2O_2 content was determined according to Sergiev et al. (1997). All chemicals used in the analyzes were of analytical grade.

All analyzes were carried out with three replicates. SPSS 22 was used in statistical analysis of the data. Comparison of means was performed using the least significant difference (LSD) test at $p<0.05$. The Pearson correlation was used to determine the relationship between the data obtained.

RESULTS AND DISCUSSIONS

It was determined that the photosynthetic pigment content of macrophyte leaves decreased with increasing phosmet concentration. *Chlorophyll-a* contents reduced by 4.0% ($p>0.05$), 16.0% ($p<0.05$) and 22.9% ($p<0.05$) under the effect of 1, 10 and 100 ppm of phosmet, respectively (Figure 1A). *Chlorophyll-b* (Figure 1B) and carotenoid (Figure 1C) contents decreased by 14.94% ($p>0.05$) and 33.67% ($p<0.05$), respectively. Similar to these findings, adverse effects of on photosynthesis and photosynthetic pigment contents in plants have been stated (Salem, 2016; Iwaniuk and Lozowicka, 2022). This affect was attributed to inhibition of chlorophyll biosynthesis by excess production of reactive oxygen species (ROS) in addition to the direct effect of pesticides (Aarti et al., 2006). Negative relationships between photosynthetic pigments (*Chl-a*, *Chl-b* and carotenoid) and hydrogen peroxide were determined (Table 1). The findings may be explained by inhibition of pigment synthesis by the phosmet and/or by accelerated degradation of pigments due to ROS generation induced by the phosmet (Mostafa and Helling, 2002).

It has been reported that pesticide toxicity causes metabolic disorders in plants (Sharples et al., 1997). Phosmet applications resulted in decrease in total carbohydrate content reaching 55.95% ($p<0.05$) (Figure 1D). Similar result was reported dimethoate, organophosphorus insecticide, applied mung plant (Seth et al., 2014). Significant decrease in total carbohydrate content *Vitis vinifera* following flazasulfuron, herbicide, application was also stated (Magne et al., 2006). Kumar (2012) also declared comparable findings in 2,4-Dichlorophenoxy acetic acid and isoproturon treated wheat (*Triticum aestivum* L.) and suggested that carbohydrate depleting affect may be involved in the toxicity of these two herbicides in addition to their main adverse effects of being synthetic auxin and acetolactate synthase inhibitor, respectively. As for phosmet, ROS elicited oxidative damage to carbohydrates may be suggested as underlying mechanisms of observed affect as supported with significant and negative correlation between total carbohydrate and hydrogen peroxide contents (Table 1).

Plant phenolic compounds, called secondary metabolites, have roles in environmental stress (Dogan and Gultekin, 2017; Cinar and Dogan, 2020). Phosmet exposure caused dose-dependant reduction in the content of total phenolic compound reaching 57.1% ($p<0.05$) (Figure 2A). Lin et al. (2022) stated decrease in phenolic acids in peppermint (*Mentha piperita* L.) following application of five insecticides (imidacloprid, pyriproxyfen, acetamiprid, chlorantraniliprole, and chlorfenapyr.) and suggested it as an adaptive response causing generation of lignin as permeability barrier to prevent absorption of insecticides.

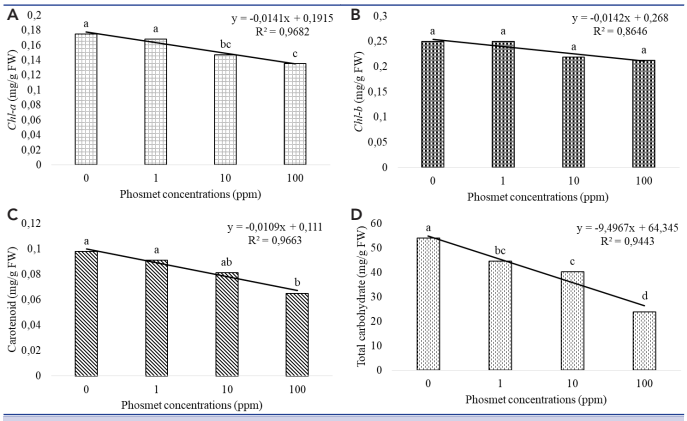

Figure 1. Photosynthetic pigments (A-C) and total carbohydrate (D) contents of *L. gibba* after phosmet applicaitons and their statistical evaluations. Means with different letters are significantly different from one another according to LSD test (p<0.05).

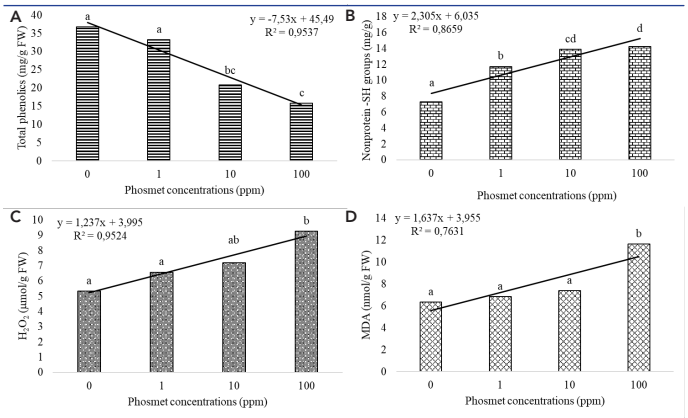

Figure 2. Total phenolic (A), NP-SH (B), H_2O_2 (C) and MDA (D) contents of *L. gibba* after phosmet applicaitons and their statistical evaluations. Means with different letters are significantly different from one another according to LSD test (p<0.05).

The non-protein sulfhydryl groups (NP-SH) showed an increasing tendency with the percentage changes of 60.8%, 90.1% and 95.5% at 1, 10 and 100 ppm concentrations (p<0.05), respectively (Figure 2B). Glutathione (GSH), the greater part of the non-protein sulfhydryl groups playing vital role in detoxification of xenobiotics, including pesticides, in plants (Yu et al., 2022). Mitton et al. (2016) determined increase in levels of NP-SH groups in alfalfa roots and soybean leaves following organochlorine pesticide dichlorodiphenyltrichloroethane (DDT) exposure. This result was referred as a general mechanism to maintain redox status via increase in low molecular weight thiols like glutathione by authors. In the present study, a significant and positive relationship between NP-SH and H_2O_2 contents were also determined after phosmet applications (Table 1). It manifests induction of antioxidant defense system as a response to phosmet triggered oxidative stress.

Pesticide induced oxidative stress either by direct action of chemical or by increase in the production of ROS have been reported (D'Souza, 2017). Phosmet applications resulted in increase in H_2O_2 level by 23.5%, 34.9% (p>0.05) and 73.5% (p<0.05) following 1, 10 and 100 mg/L concentrations, respectively (Figure 2C). H_2O_2, a non-radical, is the two-electron reduction product of oxygen and involved in oxidative degradation of lipids which is known as lipid peroxidation. 82.99% increase in MDA content was elicited by phosmet (Figure 2D; p<0.05) and correlation analysis showed that H_2O_2 and MDA contents were positively and significantly correlated (Table 1). Similarly, Dubey et al. (2015) stated rise in H_2O_2 and lipid peroxidation in barley (*Hordeum vulgare* L.) following methyl parathion (insecticide) and hexaconazole (fungicide) exposure. Nohatto et al. (2016) reported occurrence of oxidative stress manifested by increased H_2O_2 and lipid peroxidation levels in rice plants exposed to three different herbicides namely bentazon, penoxsulam and cyhalofop-butyl.

CONCLUSIONS

The determined decrease in the contents of photosynthetic pigments and total carbohydrate shows the involvement of disruption of photosynthesis and energy metabolism in the toxicity of phosmet. The elevation in occupied biomarkers of H_2O_2, lipid peroxidation and NP-SH clearly indicates phosmet elicited oxidative stress in duckweed.

Table 1. Correlation coefficients of the parameters obtained after phosmet applications.

	Chl-a	Chl-b	Car	TC	TP	NP-SH	H_2O_2	MDA
Chl-a	1							
Chl-b	0.757**	1						
Car	0.746**	0.885**	1					
TC	0.900**	0.566	0.693*	1				
TP	0.954**	0.782**	0.731**	0.843**	1			
NP-SH	-0.807**	-0.541	-0.607*	-0.798**	-0.858**	1		
H_2O_2	-0.673*	-0.307	-0.432	-0.848**	-0.628*	0.627*	1	
MDA	-0.820**	-0.631*	-0.684*	-0.888**	-0.759**	0.581*	0.765**	1

Chl-a: Chlorophyll-a, Chl-b: Chlorophyll-b, Car: Carotenoid, TC: Total carbohydrate, TP: Total phenolics, NP-SH: Nonprotein sulphydril groups, H_2O_2: Hydrogen peroxide, MDA: Malondialdehyde

*: Correlation is significant at the 0.05 level (2-tailed)

**: Correlation is significant at the 0.01 level (2-tailed)

Conflict of Interest: The authors declare that they have no competing interests.

Ethics Committee Approval: Ethics approval was not required for this study.

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