The Effects of Biopesticide on the Antioxidant Enzyme Activities of *Lemna minor*

Muhammed Atamanalp, Gonca Alak*, Özden Fakıoğlu, Arzu Uçar and Veysel Parlak

1Department of Aquaculture, Fisheries Faculty, Atatürk University, Turkey
2Department of Basic Science, Fisheries Faculty, Atatürk University, Turkey

Submission: February 07, 2019; Published: February 22, 2019

Corresponding author: Gonca Alak, Department of Aquaculture, Atatürk University, Turkey

Abstract

In this study, *Lemna minor* which were identified as suitable plant material for ecotoxicological investigations in recent years, was exposed to different levels of bio pesticides (40-80-120 μl 100 ml), for 21 days. Treatment and control groups’ plants antioxidant enzyme activities glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), glucose-6-phosphate dehydrogenase (G6PD) glutathione reductase (GR) glutathione-S-transferase (GST) and malondialdehyde level (MDA) were analysed weekly (0., 7., 14, and 21. day) at during the research. The obtained data indicated that the administration doses caused changes on the antioxidant enzyme activities and even the induction effect for some enzymes (p <0.05). The inducing effects of concentrations on antioxidant enzyme activity differed from group to group. It has also been observed with low MDA levels in the subject groups that do not cause oxidative damage.

Keywords: Biopesticide; Enzyme; MDA; Aquatic plant; Antioxidant

Introduction

It has become inevitable to use pesticides in order to get more efficiency from the unit area in agricultural activities all over the world. Initially, the use of these chemicals was welcomed and later revealed that these compounds had many negative effects on the environment and human health. Pesticides can stay around for years without degradation. Pesticides that are once transmitted to the ecosystem, cause accumulation through the aquatic nutrient chain and this accumulation is increasing in the upper levels of the food chain [1]. The use of pesticides causes serious problems on human health and environment. Pesticides effect the organism by blocking cellular processes and causing changes in the cell. Some pesticides’ residual effect lasts for days, weeks or months. These effects of the pesticides vary according to the chemical properties of the compound, the amount of concentration, temperature, humidity, pH and microorganism activities.

Increased concern about the environment and health, high consciousness on the harms of synthetic chemicals has led to intensification of studies on natural control methods of harmful livings. Biopesticides are groups of pesticides that have reduced risks compared to synthetic pesticides. Effecting in narrow area, slow moving ability and special effect shapes are the advantages of these. They are preferred since they do not make residue, remove the harmful effect without destroying the harmful living, and affect only the target harmful, limited resistant, economic, and friendly to environment and health.

Among these pesticides, azadiracthin is a natural insecticide widely used today which obtained by drying and powdering Azadirachta indica tree leaves [2]. Duckweed (*Lemna minor*) has been identified as a suitable plant material for ecotoxicological investigations in recent years [3-7]. *Lemna* absorbs its nutrients, ammonium and phosphate forms, from roots. Ammonium is an important source for duckweed. Duckweed is preferred because of its rapid growth rate, low fiber and high protein content. It is also used to increase water quality in different water reserves. Obermeier *et al.* [8] reported that *Lemna* sp. might be used for phytoremediation of low-level contamination with metals and organic xenobiotics. But some authors recommend a more detailed analysis of the development of the oxidative burst following copper exposure and of the enzymatic metabolism of pethoxamide in order to elucidate the extent of its removal from water. Under abiotic stress conditions, highly toxic and reactive molecules called reactive oxygen species (ROS) are formed in plants. These molecules disrupt the structure of proteins, lipid carbohydrates and DNA, leading to the formation of oxidative stress. The plants have antioxidant defense systems to prevent this damage, this study was planned and conducted.
to investigate the effects of azadirachtin on antioxidant defense systems of biopesticides on duckweed (*L. minor*).

**Materials and Method**

**Aquatic Plant used in the experiment**

The duckweeds are found extensive in the basins of the Pulur, Karasu and Tortum streams in the Erzurum city borders. The plants are collected fresh from these areas and transferred to the Atatürk University Faculty of Fisheries. Plant adaptation was provided in suitable aquariums at Algae Unit of faculty.

**Biopesticide used in the experiment**

azadirachtin, which is used as biopesticide, was obtained from commercial company and applied by considering the renewable static test method in 12 hours according to NOEL value.

**Trial Design**

The adapted for 4 weeks duckweeds were divided into four groups, a control group and 3 different doses of biopesticide. Three different concentrations of 40-80-120 µl of stock solution were applied to 100 ml test medium. Weekly (7, 14. and 21. days) samples were made during the 21-day trial.

**Preparing Homogenate for Antioxidant Enzyme Activity Measurements**

The samples were placed in a porcelain mortar and thoroughly crushed with a hammer, and a small amount of liquid nitrogen was added and obtained fine powder. Then KH$_2$PO$_4$ buffer solution was added to the samples, the homogenates were centrifuged at 15.000g at 15 minute 4 °C and removed from the centrifuge tube [9]. After centrifugation, the supernatant fraction was obtained, and enzyme activities were directly measured via this supernatant.

**Activity Measurements of Glucose 6-Phosphate Dehydrogenase (G6PD) Enzyme**

Nicotinamidadenin dinucleotidephosphate (NADP+) is reduced by glucose 6-phosphate dehydrogenase in the presence of glucose 6-phosphate. The formation rate of NADPH is catalyzed GSSG to glutathione reductase (GSSG-Rd) catalyzed GSH conversion, are monitored for 2 minutes [10].

**Statistical Analyses**

The data obtained from the enzyme activity measurements are given as mean ± standard deviation. (n=9). The findings were subjected to analysis of variance (ANOVA) and averages were compared using Duncan’s multiple comparison test. The importance level is taken as 0.05.

**Table 1: Lemna minor enzyme activities and MDA level effected of different levels biopesticide application**

| Day | Group | Parameters |
|-----|-------|------------|
|     | 40 µl | SOD* 0.26±0.00 |
|     | 80 µl | CAT* 0.56±0.00 |
|     | 120 µl| GPX* 0.12±0.00 |
| 0   |       | GR* 0.67±0.00 |
|     |       | GST* 0.55±0.00 |
|     |       | G6PD* 0.67±0.00 |
| 0   |       | MDA* 0.67±0.00 |

**Results and Discussion**

**How to cite this article:** Muhammed Atamanalp, Gonca Alak, Özden Fakoğlu, Azru Uçar, Veysel Parlak. The Effects of Biopesticide on the Antioxidant Enzyme Activities of Lemna Minor. Oceanogr Fish Open Access J. 2019; 9(3): 555761. DOI: 10.19080/OFOAJ.2019.09.555761
Antioxidant enzyme activities and MDA levels of treatment groups at the end of chronic treatment are given in Table 1. In the present study, different values were determined for all the treatment groups in terms of enzyme activity compared to the control. Different concentration applications showed an inducing effect on antioxidant enzyme activity and not causing oxidative damage was surveyed with low MDA levels in the corresponding groups. In terms of all the parameters examined, the statistical difference between control and treatment groups was found to be significant (p<0.05). Antioxidant enzymes have a vital prescription in the regulation of cell balance. Inductions are a consequence of the response to contaminants, and antioxidant enzyme activities and lipid peroxidation are important indicators of cell damage in toxicological studies [2,16-19].

The presence of an oxygen-rich atmosphere ensured the development of an endogenous antioxidant system that counteracts reactive oxygen species (ROS) and reactive nitrogen species (RNS) [20]. This reduction of O₂ metabolism products is controlled by the enzymatic (SOD), (CAT) and (GSH-Px) cellular defense mechanisms [21-23]. Biopesticide application generally resulted in an increase in duckweed CAT-specific activity and higher values were obtained than the control. There was a decrease in CAT activity in the 40 µL concentrations group. It can be interpreted that these changes may occur in cases where adaptation cannot be achieved and with high H₂O₂. It can also be assumed that the decline in CAT activity is due to the suppression of protein synthesis by free radicals [24].

Some enzymes are defined as "catalytically perfect" or "kinetically excellent". Examples of such enzymes include triosephosphate isomerase, carbonic anhydrase, acetylcholinesterase, catalase, b-lactamase and superoxide dismutase. When the production of O₂⁻ anion is high in the cells, the induction of the SOD enzyme takes place and the O₂⁻ anion is converted to H₂O₂. That's why; the increase in SOD activity is a result of increased O₂⁻ production. Because of the catalytic activity of many enzymes, such as dehydrogenases and oxidases, and the oxidation of many biomolecules in thiols that take place in aerobic environments, O₂⁻ anion is formed [25]. Glutathione peroxidase transfers the electrons of GSH used as a substrate in the presence of H₂O₂ to H₂O, and catalyzes the oxidation of GSH to GSSG. GPx activity increased in treatment groups compared to control. With this increase, we are thinking that oxidative stress may be induced by increasing GSSG amount, GSH / GSSG ratio and GPx activity. Again, in order to increase the oxidative toxicity resistance, it can be said that the increase in the enzyme activity occurs [2,18-19,26-27].

It is thought that in the Lemma minor affected by biopesticide, decreasing GSH level in the first few days with GSH / GSSG increasing in the next few days may be associated with newly established GSH balance or other detoxification mechanisms. It is estimated that increases in GSH levels in all time are due to an adaptive response to oxidative stress. In addition, the inability to observe significant changes in GR activity in all groups may be due to extracellular transport of GSSG in place of GSH to inhibit its cytotoxic effects. The increases in GSH level under the pollutant effect are explained by the regulation and activations that the enzymes involved in GSH synthesis can carry out to replace the GSH level [26,27]. In this study, it is thought that the decrease of GST in the pesticide-treated groups, comparison with the control is due to the increase of O₂⁻ [28]. High dose administration has severely reduced GST activity. It is known that this may be due to changes in antioxidant enzymes according to different species and tissues in response to oxidative stress [29].

GSH antioxidant defense mechanism and NADP synthesized in the pentose phosphate metabolic pathway in which G6PD and 6PGD coexist are used to produce the enzymes in this mechanism. The importance of G6PD and 6PGD in metabolism and 6PGD coexist are used to produce the enzymes in this metabolic pathway in which G6PD is a result of increased O₂⁻ production. Because of the catalytic activity of many enzymes, such as dehydrogenases and oxidases, and the oxidation of many biomolecules in thiols that take place in aerobic environments, O₂⁻ anion is formed [25]. Glutathione peroxidase transfers the electrons of GSH used as a substrate in the presence of H₂O₂ to H₂O, and catalyzes the oxidation of GSH to GSSG. GPx activity increased in treatment groups compared to control. With this increase, we are thinking that oxidative stress may be induced by increasing GSSG amount, GSH / GSSG ratio and GPx activity. Again, in order to increase the oxidative toxicity resistance, it can be said that the increase in the enzyme activity occurs [2,18-19,26-27].

How to cite this article: Muhammed Atamanalp, Gonca Alak, Özden Fakoğlu, Arzu Uçar, VeySEL Parlak. The Effects of Biopesticide on the Antioxidant Enzyme Activities of Lemma Minor. Oceanogr Fish Open Access J. 2019; 9(3): 555761. DOI: 10.19080/OFOAJ.2019.09.555761

| Treatment | Enzyme Activities EU/mg Protein | MDA Calculation | p Value | NS Value |
|-----------|--------------------------------|-----------------|---------|----------|
| 7         |                               |                 |         |          |
| 40 µL     | 0.32±0.03*                    | 0.16±0.03ab     | 0.13±0.04ab | 0.24±0.01* | 0.16±0.09bc |
| 80 µL     | 0.44±0.01a                    | 0.99±0.01a      | 0.61±0.04ab | 0.58±0.03ab | 0.37±0.09ab | 0.02±0.00bc | 0.09±0.02c  |
| 120 µL    | 1.06±0.02a                    | 0.70±0.07a      | 0.75±0.05a  | 0.59±0.05a  | 0.99±0.07a  | 0.04±0.00bc | 0.42±0.01b  |
| 14        |                               |                 |         |          |
| 40 µL     | 1.17±0.08a                    | 0.40±0.02ab     | 0.51±0.02a  | 0.63±0.03a  | 0.63±0.02a  | 0.05±0.00bc | 0.24±0.07bc |
| 80 µL     | 1.13±0.05a                    | 0.68±0.02a      | 0.31±0.01a  | 0.65±0.02a  | 0.52±0.02a  | 0.01±0.00a  | 0.08±0.01c  |
| 120 µL    | 0.81±0.04a                    | 0.61±0.02a      | 0.32±0.02a  | 0.88±0.08a  | 0.35±0.09a  | 0.02±0.00bc | 0.16±0.03b  |
| 21        |                               |                 |         |          |
| 40 µL     | 0.96±0.01a                    | 0.46±0.01ab     | 0.29±0.05a  | 0.70±0.02a  | 0.38±0.01a  | 0.02±0.00bc | 0.18±0.03c  |
| 80 µL     | 0.42±0.02a                    | 0.63±0.09a      | 0.16±0.00ab | 0.81±0.03a  | 0.32±0.07a  | 0.01±0.00a  | 0.23±0.01c  |
| 120 µL    | 0.77±0.03a                    | 0.34±0.02a      | 0.63±0.04a  | 0.54±0.07a  | 0.34±0.03a  | 0.02±0.00bc | 0.11±0.01b  |

a, b: There is no statistical difference between the averages, indicated by the same letter in the same column. *p<0.05, NS: Not Significant, Specific enzyme activities EU/mg protein, MDA is calculated as nmol/ml.
mechanism and NADP synthesized in the pentose phosphate metabolic pathway in which G6PD and 6PGD coexist are used to produce the enzymes in this mechanism. Therefore, G6PD and 6PGD are thought to be antioxidant enzymes G6PD and 6PGD enzymes have been found to decrease significantly in fish which affected by pollution. This is since these enzymes are the first enzymes of the pentose phosphate pathway and that the increase or decrease in these enzymes is not only due to exposure to pollutants but also because these enzymes are potential targets of toxic chemicals [30].

In this study, different concentrations of azadirachtin resulted in a decrease in G6PD activity. In this process, biopesticides cause O₂ production and inhibition in enzyme activity [31]. When lipid hydroperoxides resulting from lipid peroxidation break down, most biologically active aldehydes form. These compounds are either metabolized at the cell level or diffuse from the baseline domains and radiate damage to other parts of the cell. Malondialdehyde (MDA), measurable with thiobarbituric acid, is used to determine these damages. MDA is not a specific or quantitative indicator of fatty acid oxidation but correlates well with the degree of lipid peroxidation [2,18].

It has reported that the enzyme GSH-Px inhibits lipid peroxidation primarily by protecting the damaged cell, peroxypolyanionic fatty acids, short chain fatty acids, and GSH-Px enzymes using GSH instead of MDA production into hydroxyl fatty acids [32]. Based on the results obtained from the study, it has become necessary to regularly monitor the pollution in natural water environments, to determine the effects on living, to establish a database with these studies, and to determine the impact levels of pollutants harmful for aquaculture.

Acknowledgement

We would like to thank Atatürk University Scientific Research Projects Unit and the Faculty of Fisheries for their support for our project (BAP 2016/222).

References

1. Pournourmohamadi S, Farzami B, Azizi OSN, Abdollahi EM (2005) Effect of malathion subchronic exposure on rat skeletal muscle glucose metabolism. Environ Toxicol Pharmacol 19(1): 191-196.
2. Alak G, Ucar A, Parlak V, Yeltekin AÇ, Tas IH, et al. (2017a) Aessment of 8-hydroxy-2-deoxyguanosine activity, gene expression and antioxidant enzyme activity on rainbow trout (Oncorhyncus mykiss) exposed to biopesticide. Compartive Biochemistry and Physiology Part C-Toxicology & Pharmacology 205: 51-58.
3. Appenroth K, Krech K, Keresztes A, Fischer W, Kołczek H (2010) Effects of nickel on the chloroplasts of the duckweeds Spirodela polyrhiza and Lemna minor and their possible use biomonitoring and phytoremediation. Chemosphere 78(3): 216-233.
4. Leblebici Z, Aksay A (2011) Growth and lead accumulation capacity of Lemna minor and Spirodela polyrhiza (Lemnaceae): Interactions with nutrient enrichment. Water Air Soil Pollut 214(1-4): 175-184.
5. Mechora S, Stibili V, Germ M (2015) Response of duckweed to various concentrations of selenite. Enviro Sci Pollut Res Int 22(4): 2416-2422.
6. Song L, Vijver M, Peijnburg WJGM (2015) Comparative toxicity of copper nanoparticles across three Lemnaceae species. Sci Total Environ 518(5): 217-224.
7. Fakoğlu Ö, Atamanalp M (2017) Effects of glyphosate on starch accumulation, chlorophyll and enzyme activity of duckweed (Lemna minor). Acta Aquatica Turcica 13(1): 32-41.
8. Obermeier M, Schröder CA, Helmreich B, Schröder P (2015) The enzymatic and antioxidative stress response of Lemna minor to copper and a chloroacetamide herbicide. Environ Sci Pollut Res Int 22(23): 18495-18507.
9. Hou W, Chen X, Song G, Wang Q, Chi Chang C (2007) Effects of copper and cadmium on heavy metal polluted waterbody restoration by duckweed (Lemna minor). Plant Physiol Biochem. 45(1): 62-69.
10. Beutler E (1984) Red Cell Metabolism: A Manual of Biochemical Methods. In: (2 edn) Grune & Stratton, New York, USA.
11. Sun Y, Oberley LW, Ying L (1998) A simple method for clinical assay of superoxide dismutase. Clin Chem, 34(3): 497-500.
12. Aebi H (1974) Catalase. In: Bergmeyer HU (Ed). Methods of Enzymatic Analysis, Academic Press, USA, pp.673-678.
13. Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione s-transferases. The first enzymatic step in mercapturic acid formation. J BioChem 249(25): 7130-7139.
14. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72(1-2): 248-254.
15. Luo Y, Su Y, Lin RZ, Shi HH, Wang XR (2006) 2-chlorophenol induced ROS generation in fish Carassius auratus based on the EPR method. Chemosphere 65(6): 1064-1073.
16. Alak G, Atamanalp M, Topal A, Arslan H, Kocaman EM, (2013a) Effect of sub-lethal lead toxicity on the histopathological and antioxidant enzyme activity of rainbow trout (Oncorhyncus mykiss). Fresenius Environmental Bulletin 22(3): 733-738.
17. Alak G, Atamanalp M, Topal A, Arslan H, Altn S (2013b) Histopathological and biochemical effect of humic acid against cadmium toxicity in brown trout gills and muscle. Turkish Journal of Fisheries and Aquatic Sciences 13: 315-320.
18. Alak G, Yeltekin AÇ, Tas IH, Ucar A, Parlak V, et al. (2017) Investigation of 8-OHdG, CYP1A, HSP70 and transcriptional analyses of antioxidant defence system in liver tissues of rainbow trout exposed to eprinomectin. Fish Shellfish Immunal 65: 136-144.
19. Alak G, Ucar A, Yeltekin AÇ, Comaksh S, Parlak V, et al. (2018). Neuroprotective effects of dietary borax in the brain tissue of rainbow trout (Oncorhyncus mykiss) exposed to copper-induced toxicity. Fish Physiol Biochem 44(5): 1409-1420.
20. Sen CK, Packer L, Hanninen O (2000) Handbook of Oxidants and Antioxidants in Exercise. In: Amsterdam, Elsevier, USA.
21. Wickens AP (2001) Ageing and free radical theory. Respiration Physiology 126: 379-391.
22. Wohlaeb SA, Godin DV (1987) Starvation related alterations in free radical tissue defense mechanisms in rats. Diabetes 36(2): 169-173.
23. Ucar A, Ali Al-Hamdani AH, Alak G, Atamanalp M, Topal A, et al. (2012) Effects of Carboxin on Superoxide Dismutase Enzyme Activity in Rainbow trout (Oncorhyncus mykiss). BH&AD Research Journal of Biological Sciences 5(2): 883-885.
24. Palaniappan PR, Vijayasundaram V (2008) FTIR study of arsenic induced biochemical changes on the liver tissues of fresh water fingerlings Labeo Rohita. Romanian J Biophys 18: 135-144.
25. Bernabucci U, Ronchi B, Lacetera N, Nardone A (2002) Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. J Dairy Sci 85(9):2173-2179.

26. Murphy TH, Miyamoto M, Sastre A, Schnaar RL, Coyle JT (1989) Glutamate toxicity in a neuronal cell line involves inhibition of cystine transport leading to oxidative stress. Neuron 2(6):1547-1558.

27. Szkudelski T (2001) The mechanism of alloxan and streptozotocin action in b cells of the rat pancreas. Physiol Res 50(6): 537-546.

28. Matkovič B, Witas H, Gabrielač T, Szabó L (1987) Paraquat as an agent affecting antioxidant enzymes of common carp erythrocytes. Comp Biochem Physiol187(1): 217-219.

29. Ahmad F, Al SS, Shakoori A (1995) Sublethal effects of danitol fenpropatrin'a synthetic pyrethroid, on freshwater chinese grass carp, Ctenopharyngodon idella. Folia Biol (Krakow) 43: 151-159.

30. Reiter R, Tang , Garcia JJ, Munoz Hoyos A(1997) Pharmacological actions of melatonin in oxygen radical pathophysiology. Life Sci 60(25):2255-2271.

31. Topal A, Atamanalp M, Oruç E, Kirici M, Kocaman EM (2014) Apoptotic effects and glucose-6-phosphate dehydrogenase responses in liver and gill tissues of rainbow trout treated with chlorpyrifos. Tissue and cell 46(6): 490-496.

32. Nazıroğlu M, Güler M, Özgül C, Saydam G, Küçükayaz M, et al. (2014) Apple cider vinegar modulates serum lipid profile, erythrocyte, kidney, and liver membrane oxidative stress in ovariectomized mice fed high cholesterol. J Membr Biol 247(8): 667-673.

Your next submission with Juniper Publishers will reach you the below assets

- Quality Editorial service
- Swift Peer Review
- Reprints availability
- E-prints Service
- Manuscript Podcast for convenient understanding
- Global attainment for your research
- Manuscript accessibility in different formats (Pdf, E-pub, Full Text, Audio)
- Unceasing customer service

Track the below URL for one-step submission

https://juniperpublishers.com/online-submission.php