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Oxidative stress in Elodea canadensis and Lemna minor exposed to Calliofop 36EC

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ABSTRACT

The ability to withstand oxidant stress is critical for the survival of the organism. Many mechanisms protect cells against foreign elements and reactive oxygen species. The accumulation of intracellular pro-oxidant toxic substances is prevented by the action of antioxidant compounds but also by the action of antioxidant enzyme systems. In this approach we have demonstrated the capability of two aquatic plant Elodea canadensis and Lemna minor managed the oxidative stress caused by a molecule based herbicide diclofop methyl, the treatment of aquatic plants was done at the laboratory on two treatment durations 7 and 21 days in beakers of 500ml of distilled water at how we add a diclofop Methyl different concentrations: 0(control), 35, 70 and 140 µg. to prove ownership of these macrophytes fought against oxidative stress by producing specific enzymes we carried out the determination of biomarkers such activity-ascorbate peroxidase (APX), Guaiacol-peroxidase activity, the activity Glutathione S-transferase (GST), and lipid peroxidation malondialdehyde (MDA). The results show that oxidative stress in Elodea canadensis and Lemna minor was neutralized by the induction of antioxidant production. (APX) were significantly increased in the duckweed after 7 days and 21 days, in Elodea canadensis was recorded a significant increase ($p \leq 0.05$) in the APX and those at two treatment times. (GPX) showed a highly significant increase ($p \leq 0.001$) after 7 and 21 days in both macrophytes, there is induction of (GST), for LM was significant ($p \leq 0.05$) after 7 and 21 days and for EC induction was very significant ($p \leq 0.01$) the two durations of treatment, (MDA) in LM and EC increased very significantly compared to control ($p \leq 0.01$) after 7 and 21 days.

Key words: Ascorbate-peroxidase activity, Guaiacol-peroxidase activity, malondialdehyde, Glutathione S-transferase, Oxidative stress, Lemna minor, Elodea canadensis, Diclofop methyl.

Introduction

The use of phytosanitary products in agricultural activities is the major cause of pesticide contamination in water due to their being continuously discharged into aquatic environments via surface runoff (Kloeppel and *et al*., 1997). It is well-known that these pollutants have adverse effects on aquatic organisms and ecosystems (He and *et al*., 2005). To minimize the impact of this pollution, it is important to develop innovative technologies to clean the contaminated water. Existing techniques for remediation of such polluted water are based on physical and/or electrochemical treatments (Jia and *et al*., 2006). These methods are sometimes effective, but always expensive (Business Publishers Inc., 2004) In the past ten years, the use of plants to remediate contaminated soils and water (so-called phytoremediation) has gained popularity as a cost-effective, environmentally friendly and efficient in situ technology for a variety of pollutants (Cunningham *et al*., 1995; He and *et al*., 2005; Pilon-Smits, 2005 Dhir *et al*. 2009) and, among them, many pesticides (Schröder and Collins 2002; De Carvalho *et al*. 2007; Dosnon-Olette *et al*. 2009; Moore and *et al*. 2009). Indeed, some plants have a natural ability to absorb and hyperaccumulate trace elements in their tissues (Zayed and *et al*., 1998; Qian and *et al*., 1999; Gao and al., 2000). Aquatic plants have great potential to function as on-site biosinks and biofilters of aquatic pollutants because of their abundance and limited mobility (Gao and *et al*., 2000). They have been successfully used to sequester selected heavy metals and nutrients through their root systems and by uptake through their plant bodies (Bragato and *et al*., 2006). Lemna minor (L. minor), Elodea canadensis (E. canadensis) are widespread, free-floating, easy to cultivate and fast-growing aquatic macrophytes.

Many experiments conducted on the first two showed their very good accumulation capacities and their efficiency in the phytoremediation of water contaminated with heavy metals and nutrients (Wahaab and *et al*., 1995; Ka¨hko¨nen and Manninen, 1998). However, little or no data are available on the effectiveness of these two plants for the phytoremediation of pesticides (Gao *et al*., 2000; Tront and Saunders, 2006). Because plants are static and live in a competitive and sometimes hostile environment, they have evolved mechanisms that protect them from environmental abiotic stress, including the detoxification of xenobiotic compounds (Sandermann 2004). Plant metabolism is extremely diverse and can be exploited to treat recalcitrant pollutants, not degradable by bacteria or fungi. Plants may therefore be considered as "green livers", acting as an important global sink for environmental pollutants, in many cases detoxifying them (Sandermann 2004). Since there is often an analogy of structure between xenobiotics and plant secondary metabolites, it is likely that the

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metabolism of xenobiotics uses at least partially secondary metabolic pathways (Singer and *et al*. 2003). It is known that plants often metabolize xenobiotic pollutants by three sequential steps (Sandermann 1992, 1994; Coleman *et al*. 1997): (1) Phase I involves conversion/activation (oxidation, reduction, and hydrolysis) of lipophilic xenobiotic compounds (Komives and Gullner 2005); this transformation is mostly catalyzed by cytochrome P450 monooxygenases P450, (Pflugmacher and *et al*. 1999). (2) Phase II involves conjugation of xenobiotic metabolites of phase I to endogenous hydrophilic molecules such as sugars, amino acids, and glutathione (GSH) (Coleman and *et al*. 1997; Dietz and Schnoor 2001); this conjugation is mostly catalyzed by glucosyltransferases (GTs) and glutathione S-transferases (GSTs) (Pflugmacher and *et al*. 1999; Loutre and *et al*. 2003). (3) Phase III is a storage/ excretion phase, in which modified xenobiotics are compartmentalized in vacuoles or getting bound to cell wall components such as lignin or hemicellulose (Coleman and al. 1997; Dietz and Schnoor 2001; Eapen *et al*. 2007).

In this study we demonstrated the influence of the herbicide Calliofop 36 EC on both aquatic plants: Elodea canadensis (Michaux, 1803) and the duckweed Lemna minor, these two plants are known for their role in xenobiotic accumulation and filtration of wastewater, this herbicide was tested in vitro conditions has two treatment times 7 and 21 days on the detoxification enzymes in macrophyte study, Ascorbate-peroxidase activity (APX), Guaiacol-peroxidase activity (GPX), malondialdehyde (MDA), Glutathione S-transferase (GST) ,on EC and LM .

Material And Methods

Experimental material:

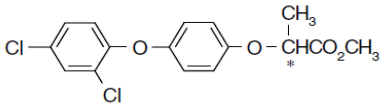
Elodea canadensis, is an aquatic species and submerged oxygenating pond or aquarium, a long, thin stems with small leaves translucent, tiny purple flowers floating green, dicotyledonous family of hydrocharitacées native to North America, it grows excessively and is very durable. (Müller., 2001).

Lemna minor, commonly known as duckweed , an aquatic macrophyte is small vascular belonging to the family Lemnaceae. The members of this family of angiosperms are monocots floating on the surface of calm water or just below (Hillman, 1961).

The herbicide used:

The herbicide used is the Calliofop 36 EC it is a product ARYSTA LifeScience (active ingredient: diclofop-methyl). Diclofop-methyl active ingredient is a herbicidally active. It is an inhibitor of the enzyme ACCase (acetyl-CoA carboxylase). This is an anti-grass, which is essentially through leaves. The active ingredient was developed in the 1970s by Hoechst AG. Since 31/12/2001, she fell into the public domain after being owned by Agrevo (then Bayer CropScience).

Chemical structure of diclofop-methyl (X. Gu *et al*., 2010)



Family: aryloxyphenoxy-propionates. Chemical formula: $C_{16}H_{14}Cl_2O_4$

Synonym: Methyl 2 - (4 -(2,4-dichlorophenoxy) phenoxy-propionate, 2-[4-(2,4-dichlorophenoxy)-phenoxy-propanoic acid methyl ester

Performing the test:

To test the effect of the herbicide Calliofop 36 EC on the Elodea Canada and the duckweed Lemna minor, we chose the following doses, 0 (control), 35, 70 and 140 ug product with three replicates per treatment at 7 and 21 days.

Determination of Biomarkers

Determination of activity-ascorbate peroxidase (APX):

The spectrophotometric determination of ascorbate peroxidase activity was performed following the protocol adopted by Nakano and Azada (1987).

Determination of Guaiacol-peroxidase activity (GPX):

Activity guaiacol peroxidase (GPX) was determined spectrophotometrically (Jenway 6300 spectrophotometer) at 470 nm using the technique of Hiner *et al*. (2002).

Determination of activity Glutathione S-transferase (GST):

Measurement of glutathione S-transferase is achieved by the method (Habig and al., 1974).

Determination of malondialdehyde (MDA):

Lipid peroxidation was estimated by changing the content of malondialdehyde (MDA) determined by the method described by Alia and *et al*., 1995.

Statistical analysis:

The results were analyzed statistically using the Minitab for Windows 13.31 (X, 2000). The data are represented by the mean plus or minus the standard deviation (m ± s). Means were compared in pairs by the Student t test. An analysis of variance with two criteria (dose, time) was performed. The significance level was $p \leq 0.05$.

Results:

Influence of herbicide on antioxidative enzymes activity:

Activity-ascorbate peroxidase APX:

Figures 1 and 2, indicate a highly significant ($p \leq 0.001$) stimulation of Activity-ascorbate peroxidase APX treated with the control input to 7 days of treatment plants for L. minor, and for the duration of 21 days we notice a significant ($p \leq 0.05$) increase in duckweed control, and after treatment by the molecule Calliofop 36 EC in Elodea canadensis is a significant ($p \leq 0.05$) increase in contribution to the control and after 7 and 21 days of treatment, These results were confirmed by the Student T test, ANOVA analysis for the two classification criteria for EC shows that it existed differences are highly significant($p \leq 0.001$) for the time factor and very significant ($p \leq 0,01$) factor for the dose, on the duckweed and ANOVA reveals highly significant($p \leq 0.001$) differences for the two factor time and dose ,and very significant ($p \leq 0,01$) for interaction dose /time.

In Lemna minor:

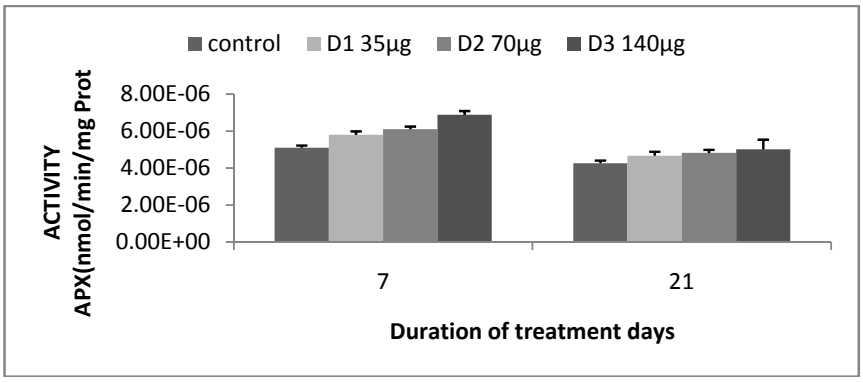


Fig. 01: Effects of Calliofop 36 EC on activity-ascorbate peroxidase (APX) in *Lemna minor* (m±s ; n=3).

In Elodea Canadensis :

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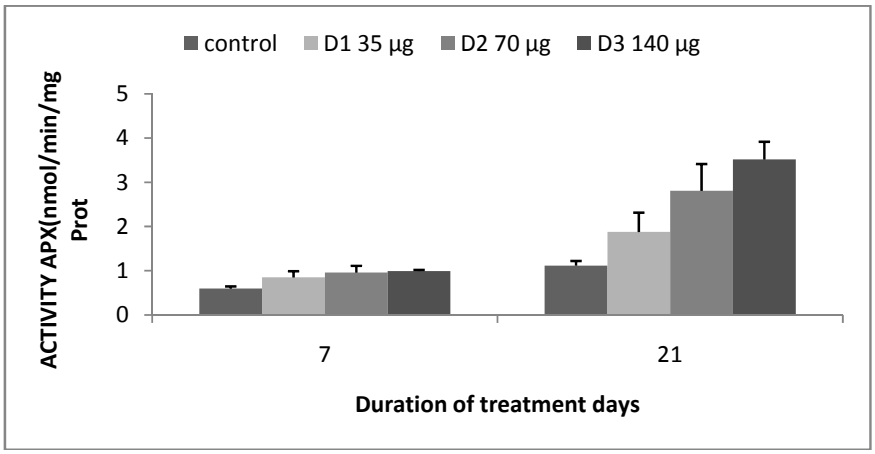

Fig. 2: Effects of **Calliofop** 36 EC on **activity-ascorbate peroxidase (APX) in *Elodea Canadensis* (m±s ; n=3).**

Activity Glutathione S-transferase GST:

The results of monitoring activity Glutathione S-transferase GST recorded for E. canadensis and L. minor shown in Figures 3 and 4, show that it increases significantly ($p \leq 0.05$) in the duckweed for D3 = 140 mcg Calliofop 36EC after 7 days of treatment, and increases very significantly ($p \leq 0,01$) for D2 = 70 mcg of herbicide product and highly significant($p \leq 0.001$) for the D3 and 21 days after this treatment, the activity Glutathione S-transferase (GST), on the plant *Elodea canadensis* has undergone an increase very significantly($p \leq 0,01$) during the 2 treatment 7 and 21 days and this for the D3, these results have been confirmed by the Student t test and ANOVA analysis showed highly significant ($p \leq 0.001$) differences for the two macrophytes under treatment with xenobiotic factors and on this dose, the interaction time and dose / time.

In Lemna minor:

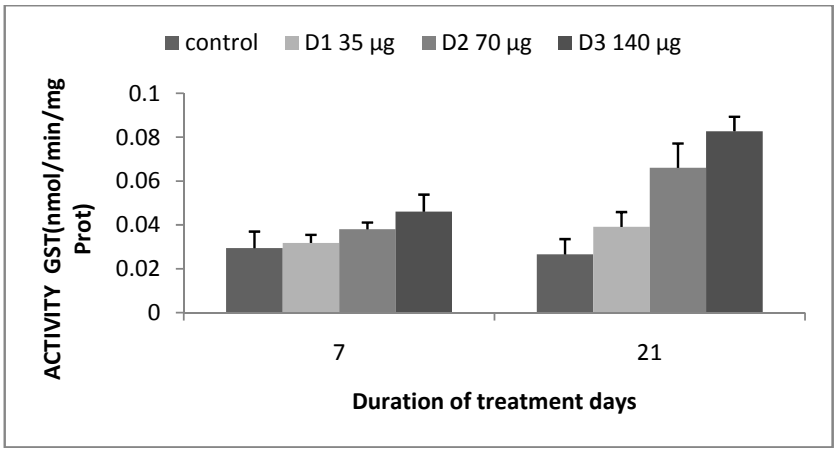

Fig. 3: Effects of Calliofop 36 EC on activity Glutathione S-transferase (GST) in *Lemna minor* (m±s ; n=3).

In Elodea Canadensis:

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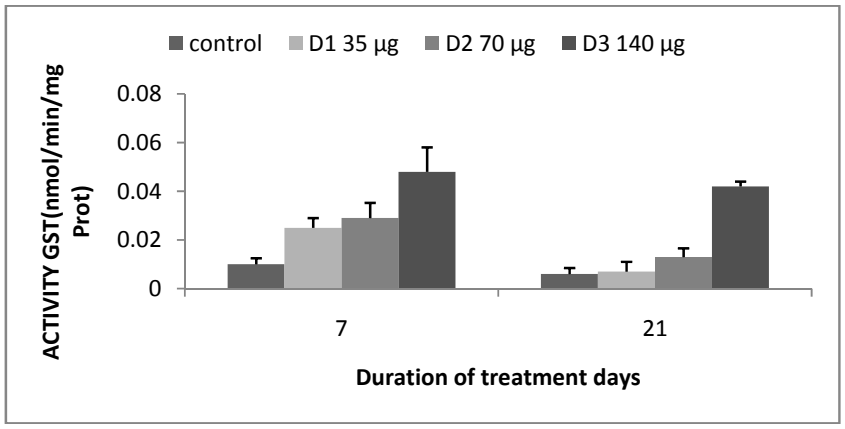

Fig. 4: Effects of Calliofop 36 EC on activity Glutathione S-transferase (GST) in *Elodea Canadensis* (m±s ; n=3).

Guaiacol-peroxidase activity GPX:

The highly significant ($p \leq 0.001$) increase in Guaiacol-peroxidase activity GPX recorded after exposure to the herbicide in the duckweed is noticed after the two times of exposure to xenobiotic 7 and 21 days for Elodea canadensis and there was a highly significant increase on the treatment after 7 days and 21 days after treatment there was a decrease by 7 days after treatment intake and increased very significantly ($p \leq 0{,}01$) contributed to the control, ANOVA indicates that differences recorded in two macrophytes on the variation of the GPX activity are highly significant($p \leq 0.001$) difference for the 2 dose and time factors and the interaction dose / time.

In Lemna minor:

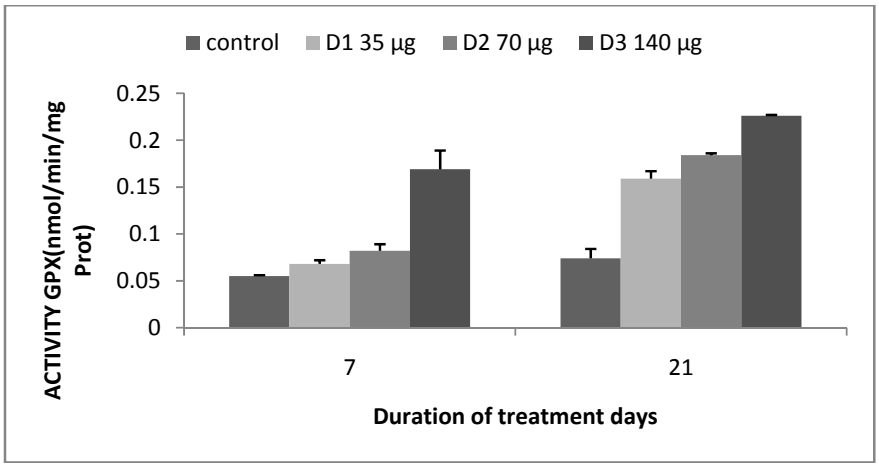

Fig. 5: Effects of Calliofop 36 EC on Guaiacol-peroxidase activity (GPX) in *Lemna minor* (m±s ; n=3).

In Elodea Canadensis:

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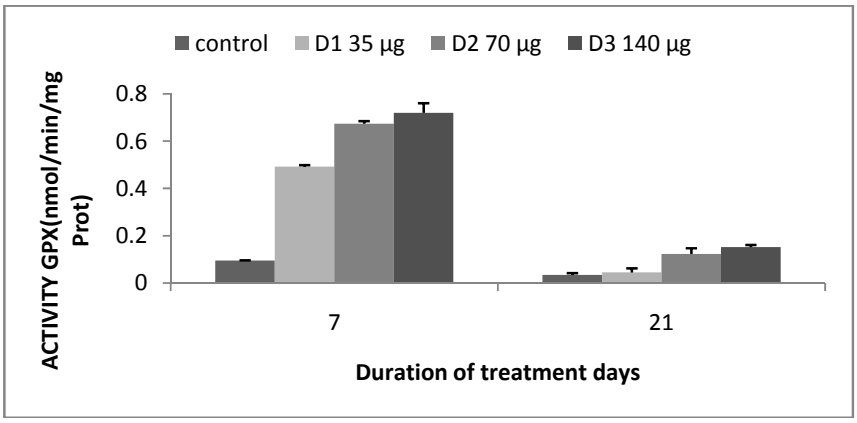

Fig. 6: Effects of Calliofop 36 EC on Guaiacol-peroxidase activity (GPX) in *Elodea canadensis* (m±s ; n=3).

Malondialdehyde MDA:

Figures 7 and 8 show the graphs found for lipid peroxidation MDA registered with *Lemna minor* and *Elodea canadensis* treated with Calliofop 36EC those below show that MDA levels increased highly significantly($p \leq 0.001$) in duckweed after 7 days of treatment and significantly($p \leq 0.05$) after 21 days of treatment, and regarding changes in MDA levels at EC we note that this rate has increased very significantly($p \leq 0{,}01$) after 7 days of treatment and for the duration of 21 days there was a significant ($p \leq 0.05$) decrease by the control input, ANOVA for it showed that differences in lipid peroxidation were highly significant($p \leq 0.001$) for two factors dose and time for *Lemna minor* and *Elodea canadensis* and significant($p \leq 0.05$) interaction for dose / time.

In Lemna minor:

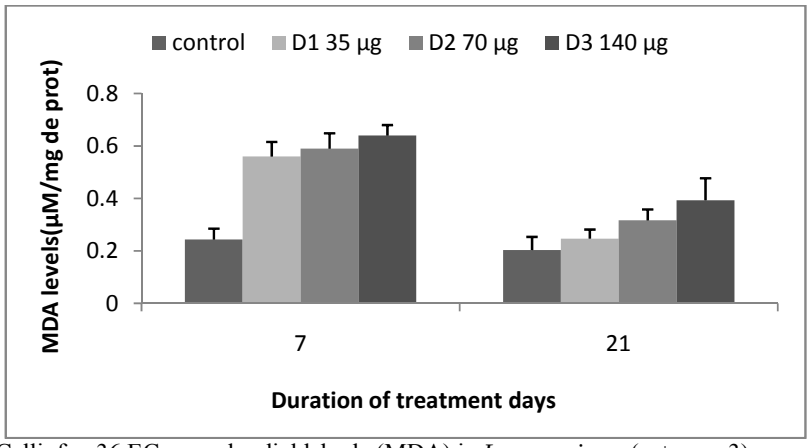

Fig. 7: Effects of Calliofop 36 EC on malondialdehyde (MDA) in *Lemna minor* (m±s ; n=3).

In Elodea Canadensis :

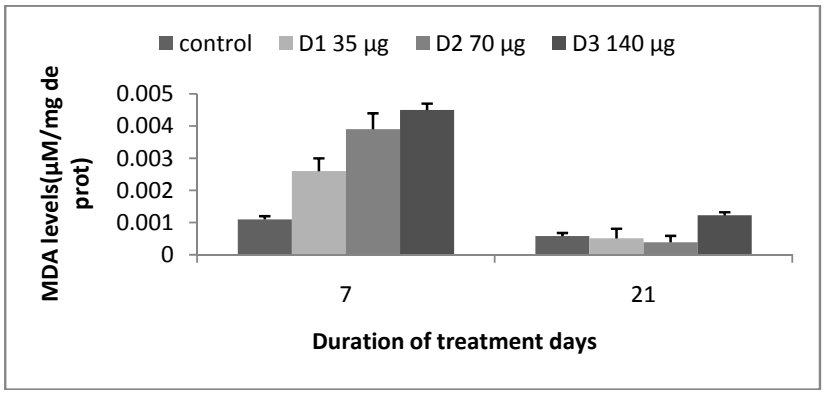

Fig. 8: Effects of Calliofop 36 EC on malondialdehyde (MDA) in *Elodea canadensis* (m±s ; n=3).

Discussion:

The resistance by detoxification corresponds to the use of enzymes which exist in the plants and their role is to protect cells by metabolizing products xénotoxiques (Eerd *et al*, 2003). Herbicides have certain chemical groups common with those products xénotoxiques and are recognized as such (Yuan and *et al*, 2007). In resistant and susceptible plants, some of these enzymes is capable to carry out the degradation of herbicides. Resistant plants show activity of degradation than that of susceptible plants, resistant plants are the only ones capable of degrading the herbicide quickly enough to prevent it has time reaching its target. (Eerd and *et al*, 2003)

The pesticides may act as xenobiotics and induce abiotic stresses in plant tissues (C.A. Moldes *and et al* ., 2008, H. Teisseire *and et al*., 1998, W. Yu *and et al.,* 2007). In the present study induction of activity of such enzymatic antioxidant as APX suggested, that the herbicide evoked oxidative stress within duckweed tissues and *Elodea Canadensis*. Besides, intensity of the antioxidative responses connected with increase significantly of APX activity was rather dependent on treatment duration, together with the herbicide elevated concentration. The authors concluded that the tolerance was a result of accumulation of antioxidants protecting membrane lipids against peroxidation. Plant enzymatic antioxidant system is composed of CAT, SOD, GPX, APX, ascorbate oxidase (AO), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR) and GST (M. Goyal *and et al*., 2010).

Besides the above antioxidative enzymes, glutathione and glutathione- associated metabolisms provide another line of defence for the protection of plants from oxidative and other forms of stress. Among them, glutathione-S-transferase (GST) plays a critical role in defending the organism against reactive electrophiles by removing them through conjugation with GSH (P. Maher., 2005). In addition, GST is ubiquitous multifunctional proteins involved in the detoxification of toxicant, which catalyses the conjugation of GSH to various electrophilic substrates (W.M. Huang *and et al*., 2008). In our experiment, the elevation significantly of GST suggested that the detoxification appears to proceed via glutathione conjugation by GST. When the highest concentration of Calliofop 36EC for two macrophytes, the damage extent of ROS was beyond the detoxification of GST.

Lipid peroxidation, which diminishes the integrity of cells and organelle membranes, is one of the most significant effects of heavy metals in plants (Schutzendubel A *and et al*., 2002), (Singh S *and et al*., 2006) Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequent tissue damage (H. Ohkawa *and et al*., 1979). Increased MDA level is considered a general indicator of oxidative stress (Cho U-H, Seo N-H., 2005), Increased MDA has already been found in L. minor and other species after exposure to Cd (Hou W *and et al.,* 2007), (Tkalec M *and et al.*, 2008) or Cu (Razinger J *and et al*., 2007) Our results have shown significantly higher MDA in all exposed plants as early as day 7. The highest MDA content found in plants treated with the largest concentrations of the herbicide, while it dropped in plants treated after 21 days of exposure to xenobiotic

The reason for the decrease of MDA is the length of treatment 21 days, after such a period caused the plant necrosis or death, so no MDA was generated anymore.

The activity of GPX varies considerably depending upon plant species and stress conditions (Gill and Tuteja.,2010), Higher peroxidase activities in water plants have been related to the tolerance to the pollutants (Lavid and *et al*., 2001; Roy and *et al*., 1992) although controversy exists in Lemna minor treated with xenobiotic, e.g. pesticides, GPX activity was not stimulated (Mitsou and *et al*., 2006; Teisseire and Vernet, 2001) or transient induction was detected (Teisseire and Vernet, 2000). In our experiments, this enzyme plays a role mainly in duckweeds and in Elodea Canadensis, there has been a highly significant ($p \leq 0.001$) increase

compared to controls and a very significant ($p \leq 0,01$) decrease in EC after 7 days of treatment .These findings are in agreement with the literature which shows that GPX up-regulation is strongly induced at the beginning of an event, and slowly decrease within time (Passardi and *et al*., 2005 and references therein

Conclusion:

The results obtained in this study highlighted the potential for accumulation of xenobiotics in aquatic plants and the capacity of these plants survived the xenobiotics in developing antioxidant enzymes exist in macrophytes that protect cells metabolizing the pesticide in question, the enzymes that play a crucial role in the detoxification tested in this experiment are GST, APX, GPX and lipid peroxidation. Induction the enzymatic activities of these was the response of plants to oxidative stress caused by the herbicide treatment administered , while so we concluded by these facts that the two aquatic plants used in this experiment *Elodea canadensis* and *Lemna minor* are effective in the extraction of pesticide wastewater by absorbing them and using enzymes metabolized defense, these two macrophytes have different tolerance thresholds in time .

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