Plant Archives Vol. 19 No. 1, 2019 pp. 111-121 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

IMPACT OF GLYOPHOSATE AND LAMBDA CYHALOTHRIN PESTICIDES ON *HYDRILLA VERTICILLATA* PLANT

Sadiqk Adhumlafta Al-Zurfi*[1], Nidal Abd Al-Hussein Albdairi[2] and Farah Raaed Abdullah Al Shamary[2]

[1]Faculty of Science, University of Kufa, Al-Najaf, Box 21, Najaf, Iraq.
[2]Faculty of Education for Girls, University of Kufa, Iraq.

Abstract

Aim of the study to test tolerance of *Hydrilla verticillata* plant to different concentrations of pesticides (glyophosate and lambda cyhalothrin) and to determine the extent of the physiological changes that occur in the plant. Collect the plant from Euphrates River and acclimation in laboratory during 15-day culture in glass aquarium three frequency exposing to different concentrations of glyophosate (2000, 6000, 12000, 24000 ppm) and lambda-cyhalothrin (33,66,166,332 ppm). Measuring total chlorophyll, chlorophylla, chlorophyll b, total protein, catalase enzyme and super oxide dismutase during (1, 3, 7 and 10 days). The current study showed a decrease in total chlorophyll and total protein content during the experiment period, while a gradual increase in the superoxide dismutase (SOD) values during the experimental period compared to the control. The values of the activity of catalase enzyme of pesticides observed a gradual decreased during experimental period. The stem of plant not affect to glyphosate while the leaf had clear affect during (3, 7 and 10 days), while affected steam and leaf of *Hydrilla verticillata* to lambda cyhalothrin pesticide. Our results indicate that glyphosate concentrations from 2000 to 24000 ppm can induce oxidative stress in *Hydrilla verticillata* and may impede metabolism processes for protein and pigments for plant. Our results suggest that the sensitivity of *Hydrilla verticillata* to higher concentrations of glyphosate and lambda cyhalothrin exposure.

Key words : *Hydrilla verticillata*, lambda cyhalothrin, glyophosate.

Introduction

Pesticide is defined as any substances or mixture of matters planned for evading, destroying, repelling or extenuating any pests (Zhang *et al*., 2016). While pesticide residues defined as any matter or mixture of material in food for man or feed for animals resulting from the use of pesticide including any determinant derivatives, such as conversion and degradation products, metabolites, reaction products and dirtiness considered to have significant toxic effect (Book, 2014).

Classified of pesticides according to action as destroying, repelling and mitigating agents. Insects and pests are getting resistant to the commercial pesticides due to over usage. Recently, pesticides have been developed which target multiple species (Speck- Planche *et al*., 2012). Obviously, exposure to pesticides poses a continuous health risk, especially in the agricultural working environment. By their very nature most pesticides show a high degree of toxicity because they are designed to kill certain organisms and thus generate some risk of detriment. Within this context, pesticide use has raised serious concerns not only of potential effects on human health, but also about effects on wildlife and delicate ecosystems (Power, 2010).

One of the mostcommonly used class of pesticides is the insecticides and herbicides, which are used for prevention and treatment of several insect infections and weeds. Of the commonly used insecticides and herbicides in Iraq are glyophosate and lambda-cyhalothrin. Although, the benefits of using pesticides to protect agricultural crops, their sequential effects on the environment and the excessive use of these substances lead to the destruction of biological diversity as many aquatic organisms are threatened by harmful pesticides for their survival (Helweg *et al*., 2003).

Author for correspondence : E-mail: sadiqk.alzurfi@uokufa.edu.iq

Aquatic plants are sensitive to water pollution and are a good indicator of their direct contact with contaminants (Mal *et al*., 2002).

In the last decade of the last century, aquatic plants were given importance because of their greater potential to treat polluted water (Solano *et al*., 2004). Where the Maxplank Institute in Germany began the adoption of aquatic plants in the 1950s in the treatment of sewage waste in wetlands (Seidel, 1976). *Hydrilla verticillata* is highly efficient in adapting to environmental conditions and has wide ranges of changes as it can grow rapidly in muddy and shallow low-light waters (Srivastava and Shrivastava, 2016). Where, it can be used as a biological indicator on stress. It is also used in phytoremediation techniques for contaminated water (Razzaq, 2017). Investigate, Al-Zurfi *et al*. (2018) the anatomical and physiological effects of cadmium in *Hydrilla verticillata* and plant response when exposed to different concentrations of cadmium metals (0.5, 1, 3, 6 mg / l) for 15 days' laboratory during days (1, 7 and 15). Found the highest values of cadmium was recorded during the experimental period in *Hydrilla verticillata* leaves and highest proportion of removal ratio of the metals was (99.4%) at 3 mg/l during the 15th day. Chemical pesticides are used extremely and without take care to manufacturing instructions. Moreover, 30% of pesticides soled in developing countries do not meet internationally quality standards (Diop *et al*., 2016). In this study, we examined the effects of different glyphosate and lambda cyhalothrin concentrations on chlorophyll pigment, total protein content and antioxidantenzyme activity and anatomy variations in *H. verticillata*. Our primary goals were to understand the adaptability of *H. verticillata* to glyphosate and lambda cyhalothrin exposure and to provide theoretical guidance for the selection of water purification plants and pesticides safety assessment.

Materials and Methods

Conducted of the experiment under laboratory conditions of light (500 Lux) and temperature (28^{0}C) from October 2017 to February 2018 as the plant collected from the Euphrates River in nylon bags after washing with river water and acclimation in laboratory during 15-day were washed numerous times with tap water then distilled water in order to remove any small invertebrate and algae (Lytle and Smith, 1995). Triplicate batch tests were conducted in glass container of dimensions (30×30×30 cm). Chosen pesticides concentrations was added in each container from prepared stock solution of glyphosate (2000, 6000, 12000, 24000 ppm) and lambda-cyhalothrin (33, 66, 166, 332 ppm). About 100 gm plant was kept in each container noticeable for the water level. The photoperiod was 12 h light: 12 h dark for all containers for duration time of 10 days. Every day, tap water (leave 5 days to remove residual chlorine) was added to maintain thesame level in each container. Measuring total chlorophyll, chlorophyll a, chlorophyll b according to Aminot and Rey (2001), total protein according to Shah *et al*. (2010) catalase enzyme activity according to Frary *et al*. (2010) and super oxide dismutase (Marklund and Marklund, 1974) during (1, 3, 7 and 10 days). Adopted in the preparation of histological slides for steam and leaf of *H. verticillata* plant on method of Thammathaworn (1996).

Statically analysis

The statistical analysis was performed with CRD design with two factors, first was concertation of pesticides and second was time of days, with three replicate. The statistical analysis was performed with Genstat (2007) was used for further statistical analyses and to determine the significance difference between treatments least significant difference (the value P>0.05).

Results and Discussion

Fig. 1 shows the total chlorophyll, chlorophyll a and chlorophyll b in *H. verticillata* that exposed to glyphosate a decrease gradually during (3, 7, 10 days) compared to the control treatment. The higher value (8.3 gm/mg) of total chlorophyll recorded at 2000 ppm concertation during 3 day, chlorophyll a higher value (1.4 gm/mg) recordedat 12000 ppm concertation during 1 day, while chlorophyll b higher value (6.9 gm/mg) at 2000 ppm during 3 day. There were significant differences between the days of the experiment. Recorded the highest values of total chlorophyll, chlorophyll a and chlorophyll b in *H. verticillata* that exposed to lambda-cyhalothrin during 1 day at 66 ppm was 19.14, 3.51 and 15.64 mg/gm), respectively (Fig. 2).

The part of plants responsible for photosynthesis is chlorophyll pigment for energy production. This pigment is found in the plastids within the plant cell (Lefsrud and Kopsell, 2007), a decrease chlorophyll levels of plants clearly suggest lower or negligible rate of photosynthesis due to heterotrophic mode of nutrition that ultimately limits the biosynthesis of protein (Pati *et al*., 2013). All factors affecting photosynthetic ability will certainly affect plant growth. Glyphosate is a sturdy cation chelator due to its carboxyl and phosphonate groups and can form complexes with nutrients in plant tissues, thus making them inaccessible for biological processes. It also inhibits the synthesis of plant secondary metabolism products,

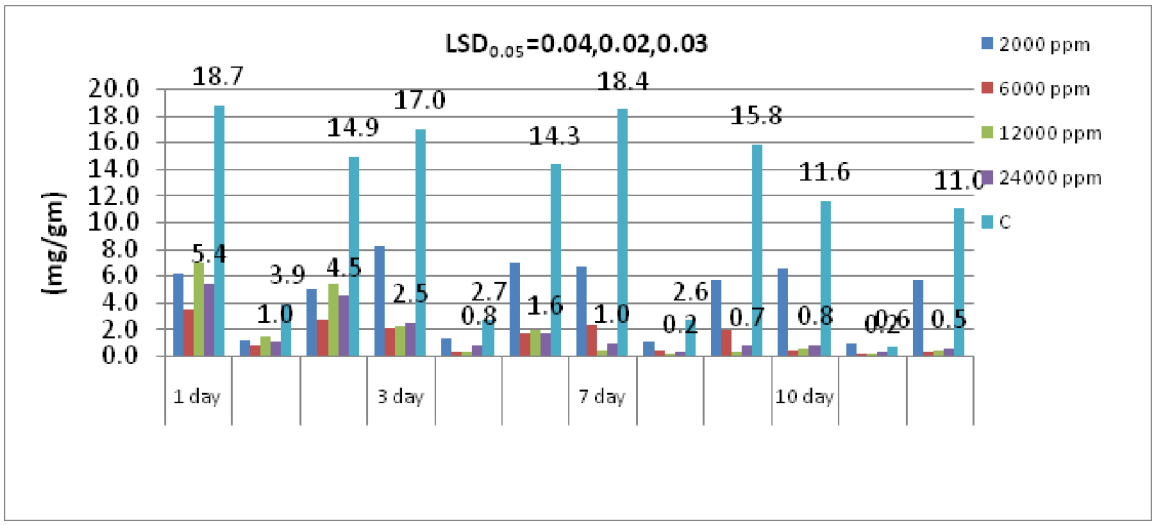

Fig. 1 : Mean total chlorophyll, chlorophyll a and chlorophyll b in *Hydrilla verticillata* that exposing to glyophosate pesticide during exposed period (mg/gm).

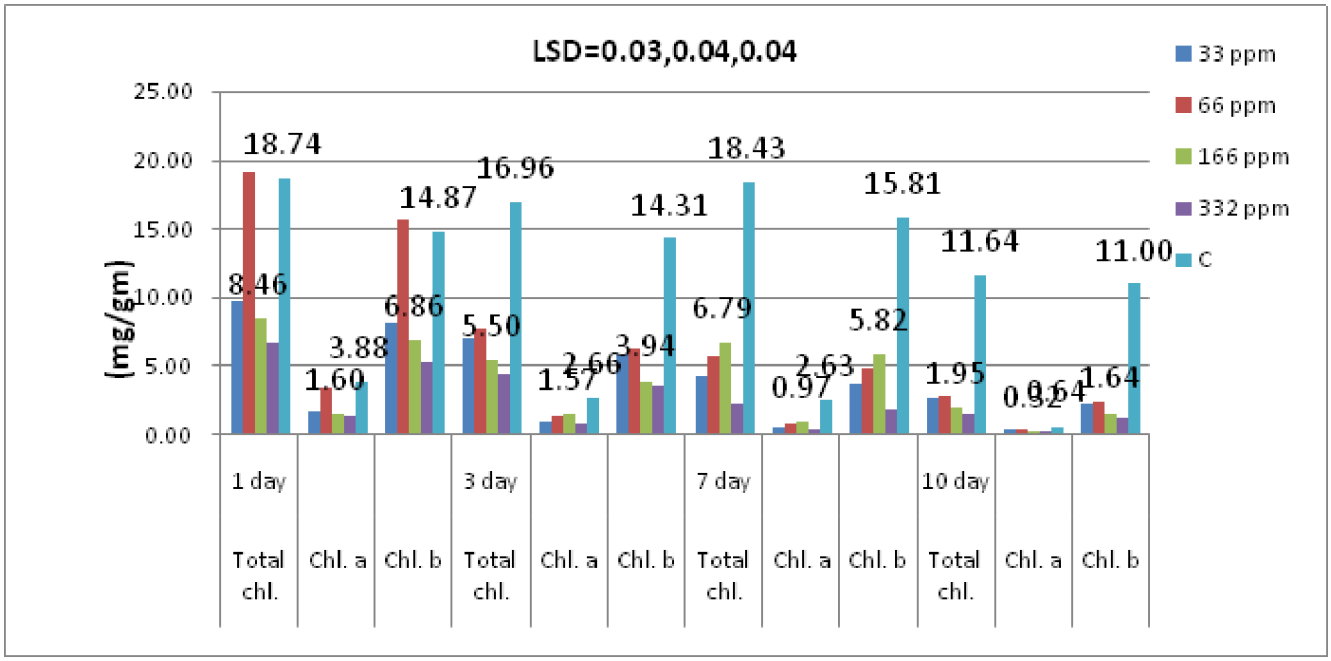

Fig. 2 : Mean total chlorophyll, chlorophyll a and chlorophyll b in *Hydrilla verticillata* that exposing to lambda-cyhalothrin pesticide during exposed period (mg/gm).

including some of the materials involved in photosynthesis such as quinones (Zhong *et al*., 2018).

Total protein of *H. verticillata* that exposed to glyphosate pesticide recorded increase values in 3 and 7 day of experimental, but decrease in 10 day (Fig. 3), while in Fig. 4 that represent total protein of *H. verticillata* that exposed to lambda-cyhalothrin pesticide show gradually decrease during 3, 7 and 10 day is due to plant stress due to the formation of reactive oxygen species, which is an oxygen-containing chemical reaction molecule such as Superoxide anion radical (O_2^-) and hydrogen peroxide H_2O_2 and hydroxyl radical (OH^-), leading to a oxidative stress that produces these compounds as transverse products during metabolism that affect in plant cells and lead to their death as well as the breakdown of protein, fat and DNA (Smirnoff, 2005). Indicating that in early stages of exposure, glyphosate did not affect soluble protein content in *H. verticillata*. It may be obtaining nutrients more easily under low glyphosate concentrations, as it was reported that low concentration oforganophosphorus pesticides may cause a dose-response relationship in algae called an "excitatory effect" (Yuan *et al*., 2014). After 3day and 7day exposure, there were no significant differences in protein content in *H.*

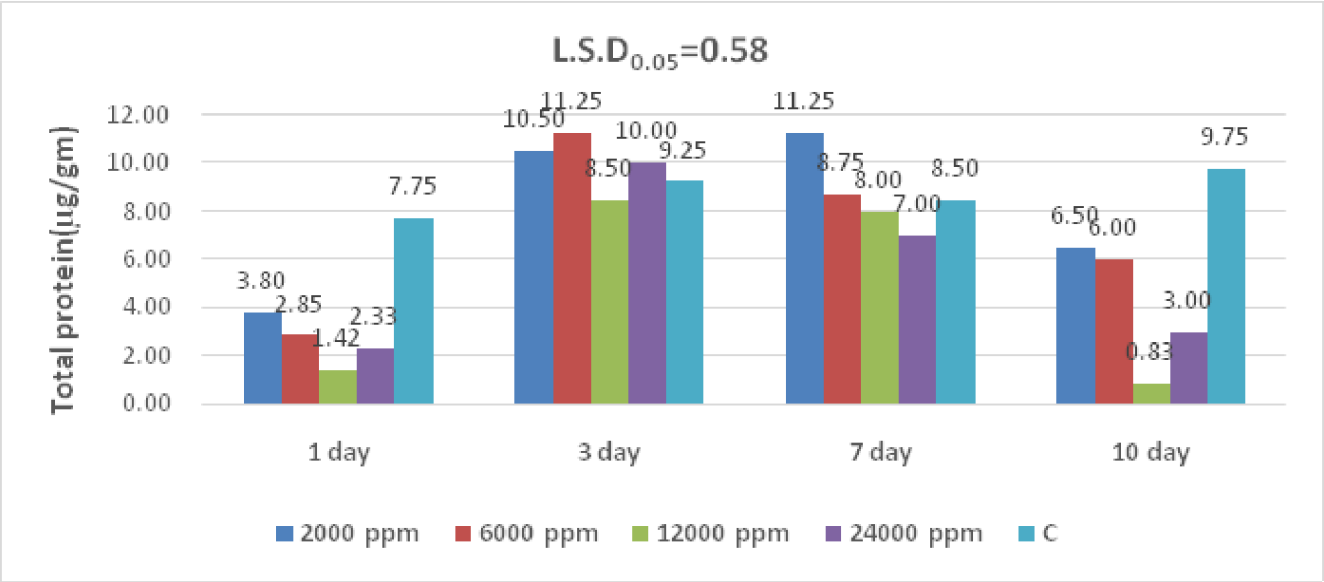

Fig. 3 : Mean total protein values in *Hydrilla verticillata* that exposing to glyophosate pesticide during exposed period (µg/gm).

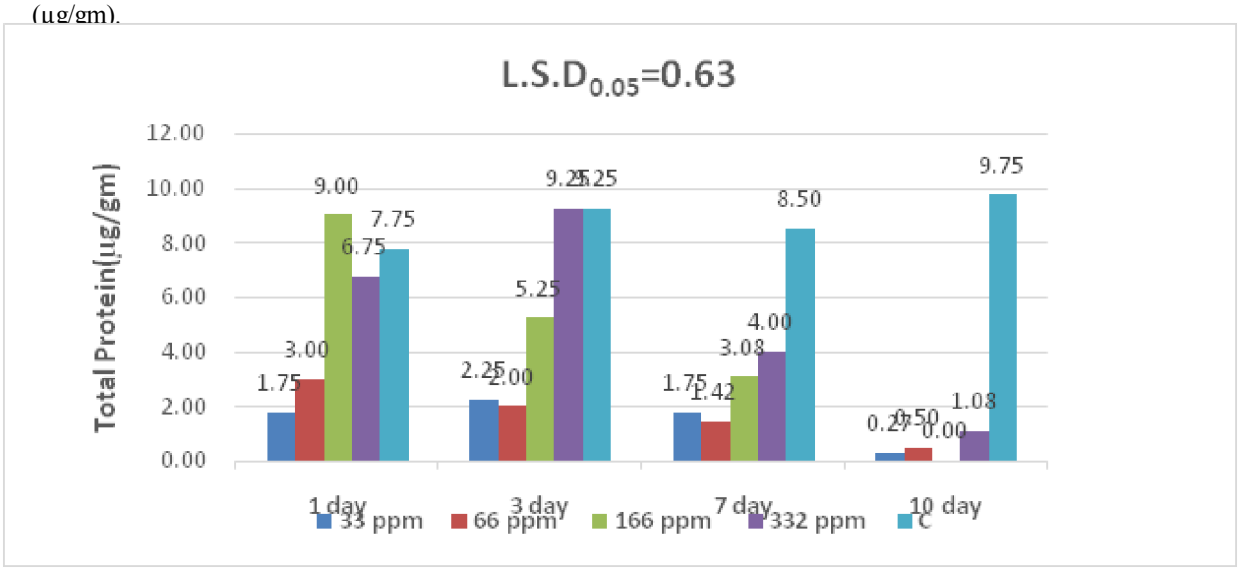

Fig. 4 : Mean total protein values in *Hydrilla verticillata* that exposing to lambda-cyhalothrin pesticide during exposed period (µg/gm).

verticillata, possibly because the plant antioxidant enzymes were activated, maintaining organism stability. However, with increased concentrations of glyphosate, *H. verticillata* protein content decreased gradually at 6000, 12000 and 24000ppm was significantly. Reduction in protein content of *H. verticillata* leaves under high glyphosate concentrations may be due to breakdown of soluble proteins or due to increased activity of protease or other catabolic enzymes, which were activated and destroyed the protein (Singh *et al*., 2005).

Mohan and Hosetti (1998) found a reduction in the protein content of *Lemna minor* treated with lead this was attributed to protein degradation due to the increased activity of protease. In Al-Zurfi *et al*. (2018) study found a decrease in the total protein of *Hydrilla verticillata* plant exposed to cadmium metal during the experiment period.

Catalase activity values recorded higher value in *H. verticillata* that exposed to glyphosate (9.99 unit/mg) at 2000 ppm during 3 day while lower value (0.72 unit/mg) at 24000 ppm during 7 day (Fig. 5), but in Fig. 6 catalase activity values show a gradual decrease during experimental recorded higher value (16.8 Unit /mg) at 332 ppm during 1 day while lower value (0.53 Unit/mg) at same concertation during 10 days. Show the results of statistical analysis were significant differences below the probability level ($p > 0.05$) between days and concentrations. Due to the plant's sensitive to possibly

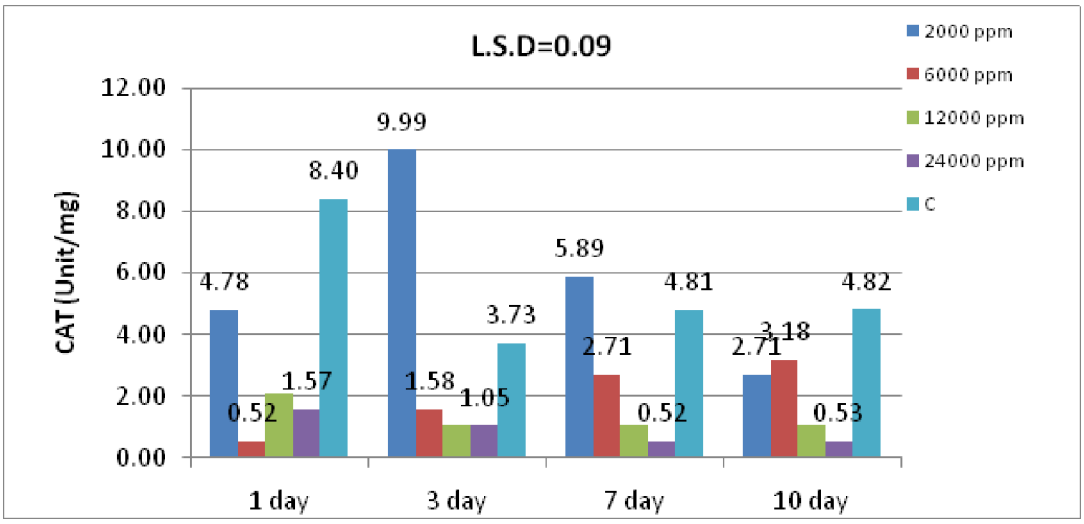

Fig. 5 : Variation in CAT activityin *Hydrilla verticillata* that exposing to glyophosate pesticide during exposed period.

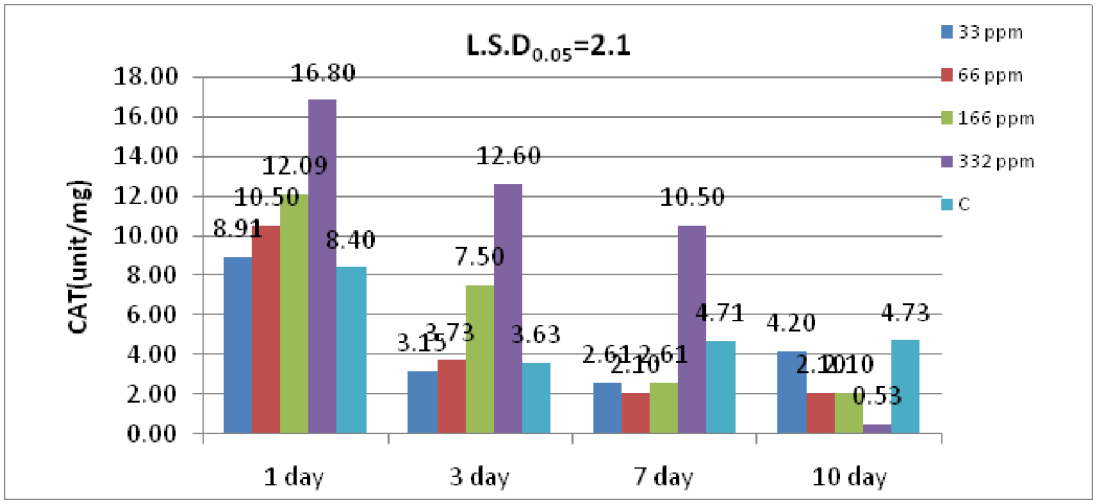

Fig. 6 : Variation CAT activity in *Hydrilla verticillata* that exposing to lambda-cyhalothrin pesticide during exposed period.

stress conditions for the period or may be to the role of pesticides in stimulating the bio-processing of antioxidant enzymes (Aravind and Prasad, 2005). CAT is important antioxidant enzyme in plants and play an important role in the removal of hydrogen peroxide. In this experiment, the CAT in *H. verticillata* did not change significantly 1day after treatment of glyphosate, which indicates that glyphosate did not cause oxidative stress over the shorter time period. After 7 day of exposure, CAT activity at 2000ppm glyphosate concentration in *H. verticillata* was significantly higher than that of the control and 24000ppm treatment, followed by a decrease with increased herbicide concentration. Gomes and Juneau (2016) studied the effects of glyphosate on *Limna minor* and found that MDA and hydrogen peroxide were significantly increased after treatment with glyphosate and that SOD, CAT and APX were significantly decreased, possibly due to inhibition of ALA synthesis.

Showed the SOD values of *H. verticillata* that exposed to glyphosate is gradual increase from the 1 day to 7 day compared to control treatment so recorded higher value (0.22 unit/mg) at 24000 ppm during 1 day and lower value was (0.04 unit/mg) at 12000 ppm during 7 day. Significant differences were recorded between experimental days and concentrations at probability level (P> 0.05) and the value of LSD = 0.06 (Fig. 7). While recorded the SOD values of *H. verticillata* that exposed to lambda-cyhalothrin pesticide gradual increase from the 3 day to 7 day and decrease in 10 day and the higher value was (0.112 unit/mg) at 33 ppm during 7 day and lower value was (0.031 unit/mg) at same concertation during 10 day (Fig. 8). Under pesticides – stress conditions, as well as excess concertation of pesticides exposure, there was an imbalance between removal and generations of ROS in plant tissues (Grataq *et al*., 2005). Antioxidant enzymes activities are very vital mechanism of plant antioxidant resistancesystem (Abbas, 2017). Our

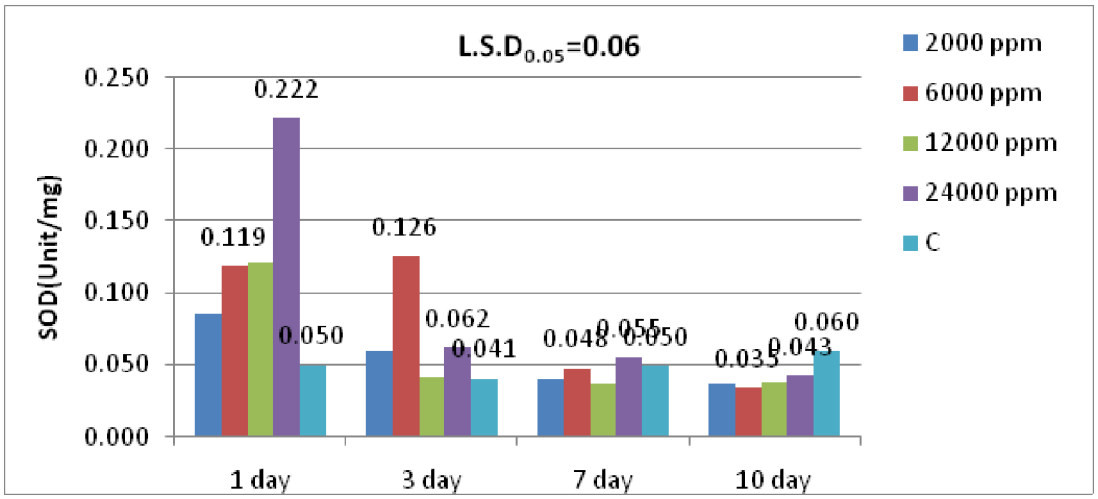

Fig. 7 : Variation in SOD in *Hydrilla verticillata* that exposing to glyophosate pesticide during exposed period.

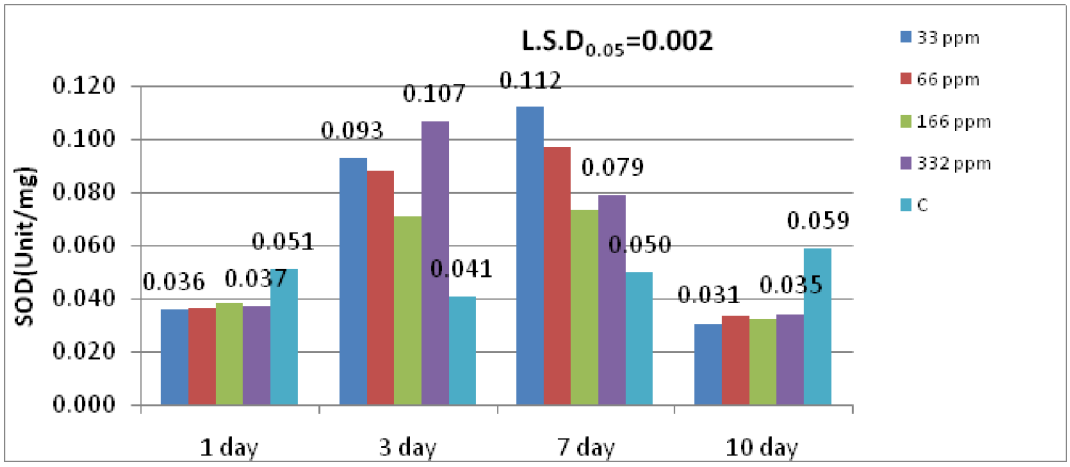

Fig. 8 : Variation SOD in *Hydrilla verticillata* that exposing to lambda-cyhalothrin pesticide during exposed period.

results showed a gradual increase in the SOD values and attributed the reasons for the high effectiveness of the enzyme may be due to the fact that the antioxidants of the enzyme have differed in their effectiveness and the ability of the plantto tolerate stress conditions (Hanfeng *et al.*, 2010). Agree the result with Zhong *et al*. (2018) and Abbas (2017) study and Al-Zurfi *et al*. (2018) study. The low efficiency of the enzyme may be due to the sensitivityof the plant to high concentrations that reduce its effectiveness and thus lead to the collection of ROS in plant tissues and increase the rate of DNA destruction (Sharma *et al*., 2012).

The image 1 shows a cross section in *H. verticillata* leaf exposed to different concentrations of glyphosate (2000, 6000, 12000 and 24000 ppm). We note in the first day the plant did not change, whereas in the third, seventh and tenth days, the plant showed yellowing, low thickness of parenchyma layer, increasing the air parenchyma layer and reduced thickness of the leaf and number of layers with increase exposed period. While, *H. verticillata* steam exposed to different concentrations of lambda-cyhalothrin (33 ppm) are shown in image 2 during the third day show degradation in air parenchyma tissues such as decreased in number and increase in size in seventh day appear degradation in vascular cylinder and parenchyma tissues, increase epidermis tortuosity with the link points of the leaf remaining intact and during 10day where the leaves were separated.

The image (3-C) shows a cross section of the *H. verticillata* steam exposed to lambda-cyhalothrin (66 ppm), showing plant during third day tortuosity in steam surface, but in 7 day, where the lack of the circumference of the vascular cylinder and increase in the epidermis tortuosity image (3-D). In tenth day the plant shows yellowing and reduced of thickness of the parenchyma layer and increase in the air parenchyma layer and degradation in thickness of vascular bundle with increased exposure time of pesticide image (3-E).

Image (4-C) showed cross section in the *H. verticillata* steam exposed to lambda-cyhalothrin (166

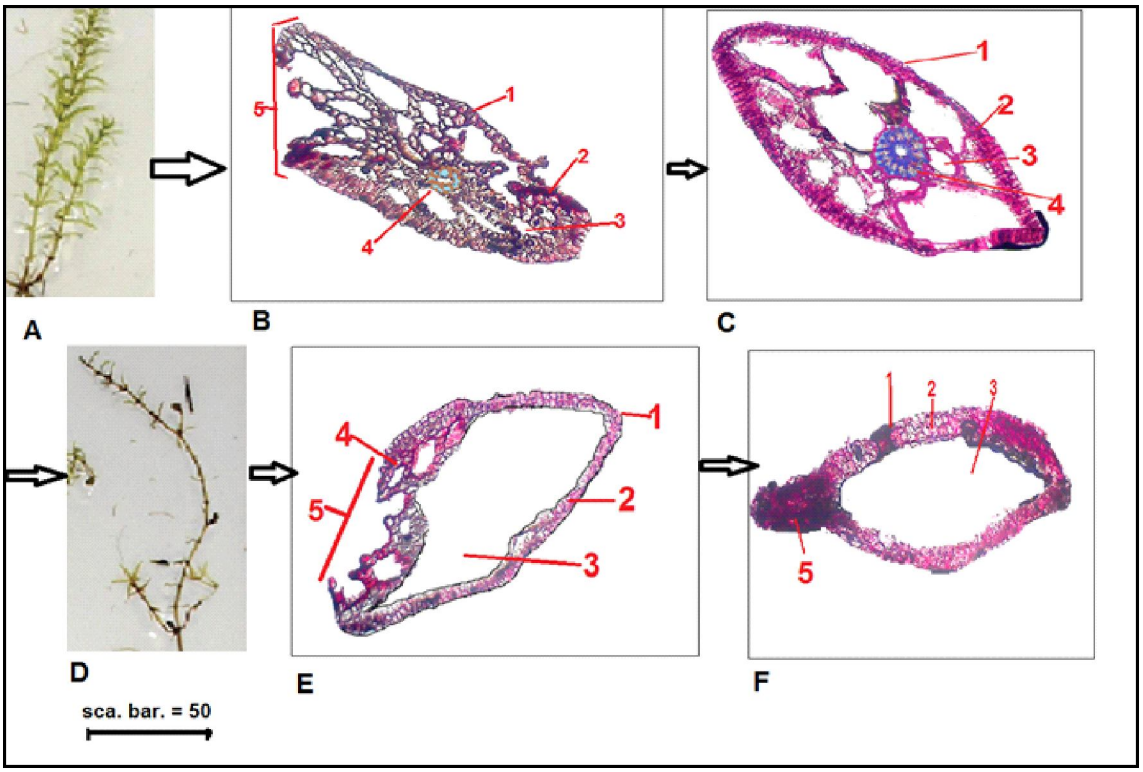

Image 1 : Transection in *Hydrilla verticillata* stem treated with lambda-cyhalothrin pesticide: a magnification force X 10, scale bars = 50 μm^{-1}, where 1 = Epidermis cells, 2 = Parenchyma tissue 3=Air parenchyma tissue, 4 = vascular bundle and 5=connective tissue of leaf in stem A) *Hydrilla verticillata* plant in 1 day B) Control C) Treatment at 33 mg / l during the 3rd day D) *Hydrilla verticillata* plant in 7 day E) Treatment at 33 mg / l during the 7day. F) Treatment at 33 mg/l during the 10 day.

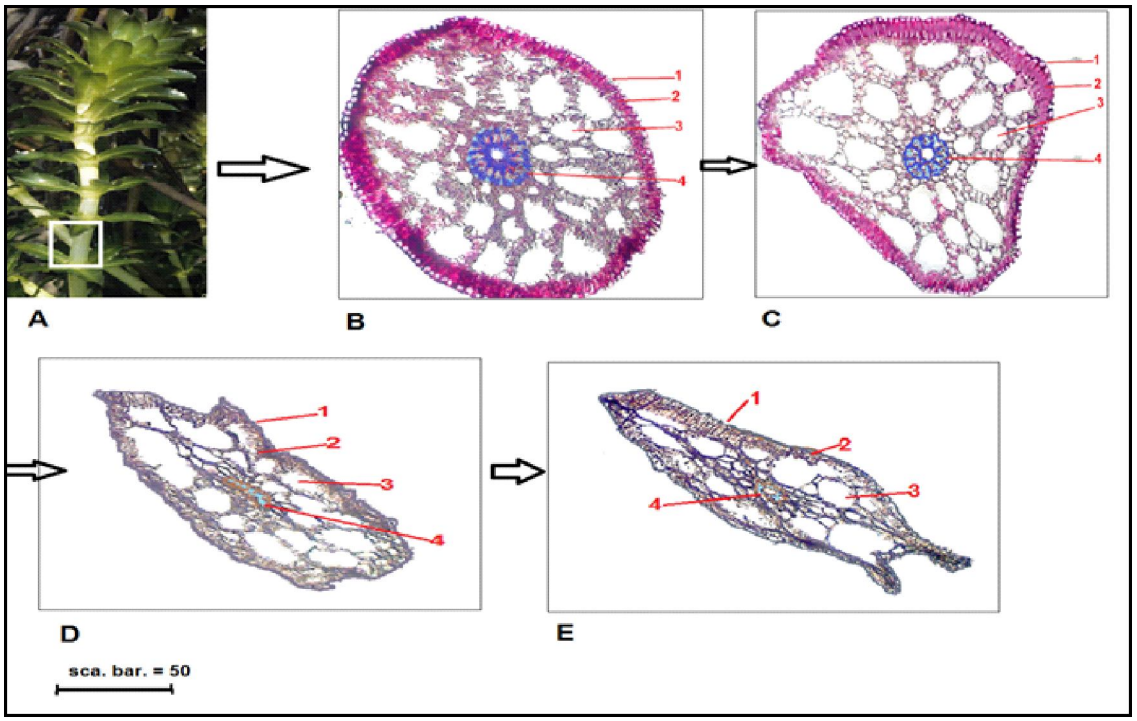

Image 2 : Transection in *Hydrilla verticillata* stem treated with lambda-cyhalothrin pesticide: a magnification force X 10, scale bars = 50 μm^{-1}, where 1 = Epidermis cells, 2 = Parenchyma tissue 3 = Air parenchyma tissue and 4 = vascular bundle A) *Hydrilla verticillata* plant in 1 day B) Control C) Treatment at 66 mg / l during the 3rd day D) Treatment at 66 mg / l during the 7day. E) Treatment at 66 mg / l during the 10 day.

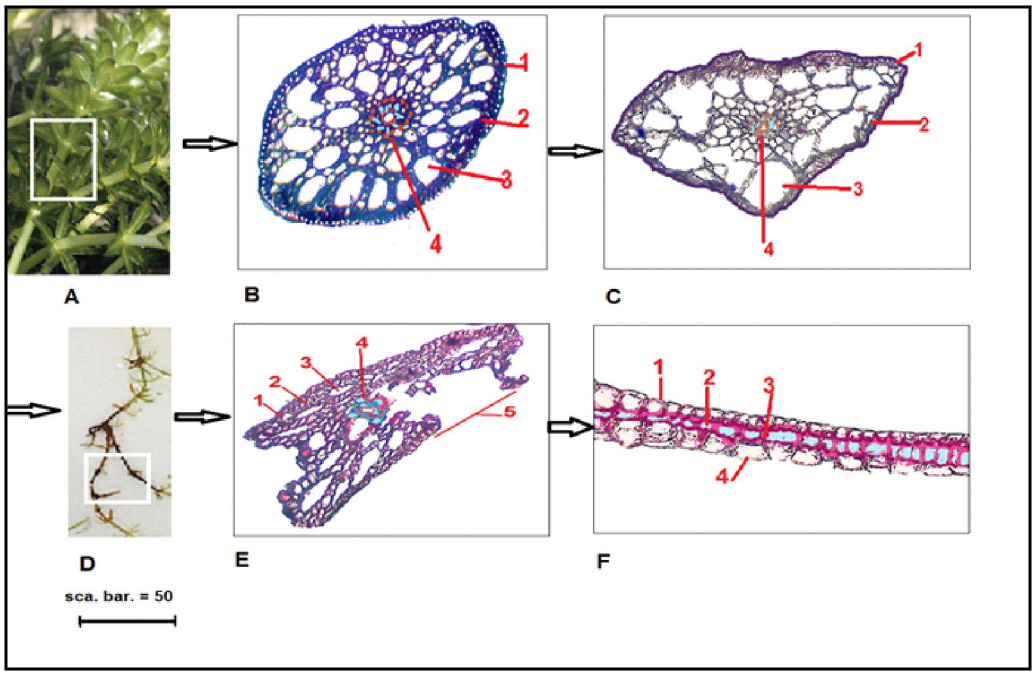

Image 3 : Transection in *Hydrilla verticillata* stem and leaf treated with lambda-cyhalothrin pesticide: a magnification force X 10, scale bars = 50 μm^{-1}, where 1 = Epidermis cells, 2 = Parenchyma tissue 3=Air parenchyma tissue 4 = vascular bundle and 5=connective tissue of leaf in stem A) *Hydrilla verticillata* plant in1-day B) Control C) Treatment at 166 mg /l during the 3rd day D) *Hydrilla verticillata* plant in Treatment at 166 mg/l during the 7day. E) Treatment at 166 mg / l during the 7day. F) Transection in *Hydrilla verticillata* leaf treatment at 166 mg / l during the 10 day.

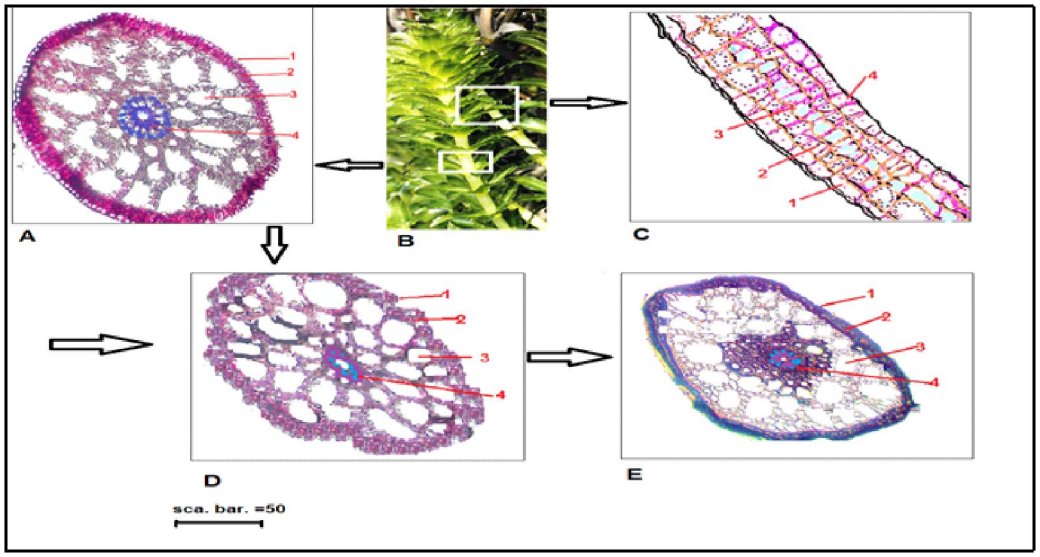

Image 4 : Transection in *Hydrilla verticillata* stem and leaf treated with lambda-cyhalothrin pesticide: a magnification force X 10, scale bars = 50 μm^{-1}, where in stem (A, D, E) 1 = Epidermis cells, 2 = Parenchyma tissue 3=Air parenchyma tissue and 4 = vascular bundle C) *Hydrilla verticillata* leaf 1 = Upper Epidermis cells, 2 = Parenchyma tissue 3 = Air parenchyma tissue and 4 = lower Epidermis cells A) Control B) *Hydrilla verticillata* plant at 332 mg / l during 1-day C) Transection in *Hydrilla verticillata* leaf treatment at 332 mg / l during the 10day D) Treatment at 332 mg / l during the 3rdday E) Treatment at 332 mg / l during the 7 day and 10 day.

ppm), as we observe the plant during third day degradation in air parenchyma tissues decrease in number and increase in size. But in seventh day, where the degradation the link points of the leaf with steam with the survival of the vascular cylinder and the parenchyma tissues remaining intact and increase in the epidermis tortuosity (image 4-E-D). During tenth day the leafs were separated (image 4-F).

While the image 5 shows cross section in the *H. verticillata* leaf and steam exposed to lambda-cyhalothrin

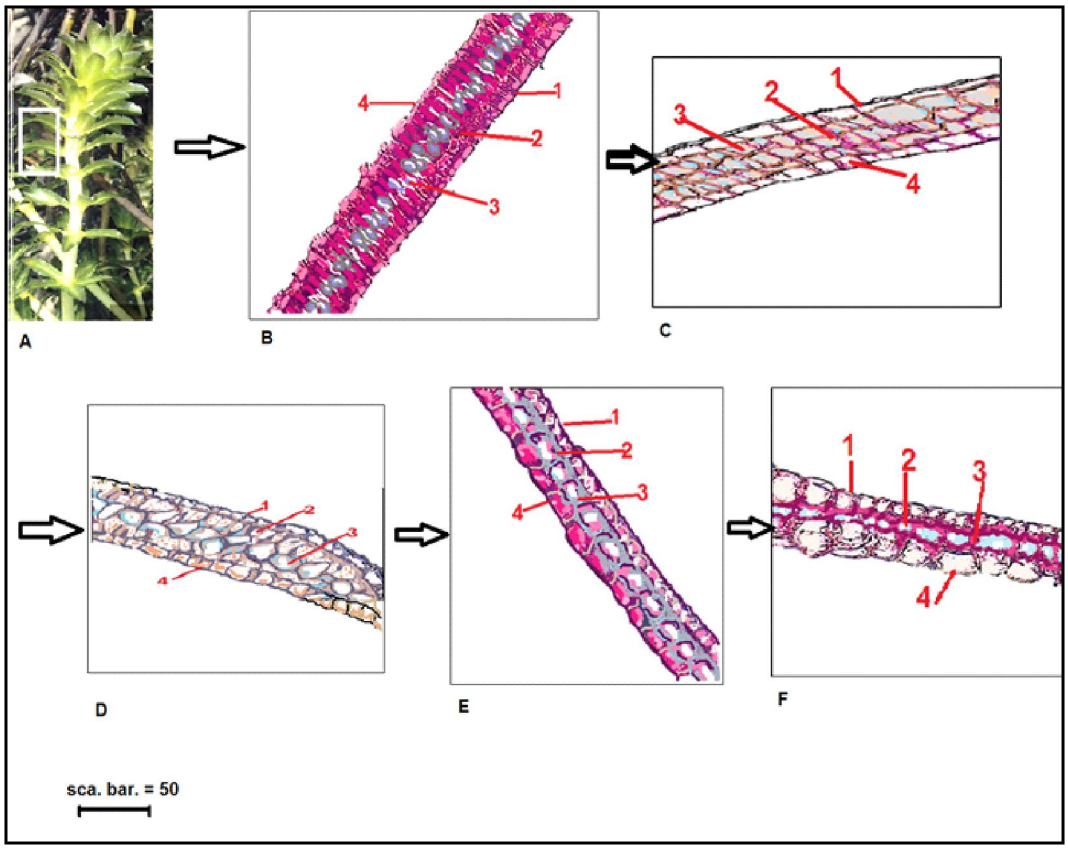

Image 5 : Transection in *Hydrilla verticillata* leaf treated with glyophosate pesticide: a magnification force X 10, scale bars = 50 μm^{-1}, where 1 = Upper Epidermis cells, 2 = Parenchyma tissue 3=Air parenchyma tissue and 4 = Lower Epidermis cells A) *Hydrilla verticillata* plant B) Control C) Transection in *Hydrilla verticillata* leaf treatment during 3 day D) Transection in *Hydrilla verticillata* leaf treatment during 7 day E) Transection in *Hydrilla verticillata* leaf treatment during 7 day F) Transection in *Hydrilla verticillata* leaf treatment during 10 day Treatment at all concentrations 2000, 6000, 12000, 24000 mg /l.

pesticide at a concentration (332 ppm), that appear not affect the leaf with increased exposure time image (5-C), during third day show epidermis surface tortuosity of steam and a decrease in size and reduction in the vascular cylinder tissue (image 5-D). During 7 day and 10 day appear degradation in photosynthesis pigments and deposition of materials in the parenchyma tissue (image 5-E).

Our results showed not affected of plant steam by glyphosate pesticide while the leaf was clear affect during (3, 7 and 10 days) of experimental where yellowing, reduced thickness of the parenchyma layer, increasing the air parenchyma layer and reduced thickness of the leaf and number of layers with increase exposed time. This is probably due to the herbicides, including the glyphosate pesticide, was used to destroy weeds. These pesticides take two ways in the effect, either impeding photosynthesis or falling leaves through cell death around the leaf neck,stress exposure leads to a decrease in the parenchyma cells thickness and this is agreed with several studies (Sandalio *et al*., 2001; Al-saadi *et al*., 2013; Al-Zurfi *et al*., 2018).

While the results of the *Hydrilla verticillata* plant that exposed to Lambda Cyhalothrin show the effect of the leaves and stems, but according to concentrations where the effect of the few concentrations on the epidermis by increasing the tortuosity with the degradation of the vascular cylinder and parenchyma tissues and the survival link points between the steam and leaf intact,whereas in the high concentrations the reverse is true where degradation link pointswiththe survival the vascular cylinder and the parenchyma tissues is intact, indicating that the pesticide works on the degradation of the correlation points in the high concentrations, may be due to possibly the plant resistance to the low concentrations their sensitivity to the high concentrations, whereas the vascular cylinder and parenchyma tissues, high concentrations may have stimulated defensive means of the plant to preserve vascular cylinder and parenchyma tissues.

Conclusion

Induction of defense mechanisms like antioxidative response and adjustment of photosynthetic performance enables the plant to protect itself when exposed to pesticides.In this study, toxic effects of two glyphosate and Lambda Cyhalothrin pesticides on *H. verticillata* photosynthetic pigment activity and oxidative damage after 10 days exposure were demonstrated. The glyphosate pesticide mainly prevented the activation of SOD, while the Lambda Cyhalothrin activation of SOD. Our results concluded the *H. verticillata* steam that exposed to glyphosate pesticide was not affected, while the leaf had a clear effect during the third, seventh and tenth days of the experiments. While that exposed to Lambda Cyhalothrin showed the affected leaves and stems, but according to concentrations.

References

Abbas, F., J. Iqbal, Q. Maqbool, T. Jan,M. Obaid Ullah, B. Nawaz, M. Nazar, M. S. Naqvi and I. Ahmad (2017). ROS mediated malignancy cure performance of morphological, optical, and electrically tuned Sn doped CeO_2 nanostructures. *AIP Advances*, **7(9)** : 095205.

Aminot, A. and F. Rey (2001). Chlorophyll a: determination by spectroscopic methods, ICES Tech. *Mar. Environ. Sci.*, **30** : 1–18.

Al-Saadi, S. A. A. M, W. M. A;-Asaadi and A. N. H. Al-Waheeb (2013). The effect of some heavy metals accumulation on physiological and anatomical characters of some *Potamogeton* L. plant. *Journal of Ecology and Environmental Sciences*, **4(1)** : 100-108.

Aravind, P. and M. N. V. (2005). Cadmium-zinc interactions in a hydroponic system using *Ceratophyllum demersum* L.: adaptive ecophysiology, biochemistry and molecular toxicology. *Braz. J .Plant Physiol.*, **17**: 3–20.

Al-zurfi, S. K. L., A. Y. Alisaw and G. A. A. Al-Shafai (2018).

Anatomical and Physiological effects of Cadmium in **18(1)** : 839–846.

Diop, A., M. D. Yérim, D. T. Diène, C. Fabrice, O. S. Serigne, K. Amaury, L. David and D. François (2016). Monitoring survey of the use patterns and pesticide residues on vegetables in the Niayes zone, Senegal. *Chemosphere*, **144** : 1715-1721.

Razzaq, R. (2017). An Environmental Friendly Technique - A Review. *Journal of Environmental Analytical Phytoremediation*, **4(2)** : 2–5. https://doi.org/10.4172/2380-2391.1000195

Frary, A., D. Göl, D. Kele, B. Ökmen, P. Hasan, O. Hasan and A. Yemenicio (2010). Salt tolerance in *Solanum pennellii*/ : antioxidant response and related QTL.

Hanfeng, X., T. Qiling and H. Chengxiao (2010). Structural and metabolic responses of *Ceratophyllum demersum* to eutrophic conditions, **9(35)** : 5722–5729.

Helweg, C., B. B. Mogensen and P. B. Sørensen (2003). Fate of Pesticides in Surface Waters. *Laboraty and Field Experiments*, **68** : 1–135.

GENSTAT (2007) Genstat Discovery Edition 3.0. Lawes Agricultural Trust Rothamsted Experimental Station, Herefordshire.

Gomes, M. P. and P. Juneau (2016). Oxidative stress in duckweed (*Lemna minor* L.) induced by glyphosate : Is the mitochondrial electron transport chain a target of this herbicide/ ?. *Environmental Pollution*, **218**. https:// doi.org/10.1016/j.envpol.2016.07.019\

Grataq, P. L., A. Polle, P. J. Lea and A. Azevedo (2005). Making the life of leaves metal stressed plants a little easier. *Funct. Plant Biol.*, **32** : 481-494.

Lefsrud, M. G., D. A. Kopsell and D. E. Kopsell (2007). Nitrogen levels influence biomass, elemental accumulations and pigment concentrations in spinach. *J. Plant Nutr.*, **30** : 171- 185.

Lytle, C. M. and B. N. Smith (1995). Seasonal nutrient cycling in *Potamogeton pectinatus* of the lower Provo river. **55(2)**: 4–9.

Marklund, S. and G. Marklund (1974). Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase, **474** : 469–474.

Mal, T. K., A. L. Adorjan and A. L. Corbertt (2002). Effect of copper on growth of an aquatic macrophytes *Elodea canadensis*. *Environ. Pollut.*, **120** : 307 – 311.

Mohan, B. S. and B. B. Hosetti (1998). Potential phytotoxicity of lead and cadmium to lemna minor grown in sewage stabilization ponds. **98(2)**.

Pati, R. (2013). Histological and biochemical changes in *Aegle marmelos* Corr . before and after acclimatization, **3(3)** : 12-18. https://doi.org/10.5376/tgmb.CITATIONS

Power, A. G. (2010). Ecosystem services and agriculture/ : tradeoffs and synergies, 2959–2971. https://doi.org/ 10.1098/rstb.2010.0143.

Sandalio, L. M., H. C. Dalurzo, M. Go´mez, M. C. Romero-Puertas and L. del Rýó (2001). Cadmium-Induced Changes in the Growth and Oxidative Metabolism of Pea Plants. *Journal of Experimental Botany*, **52(364)** : 2115–2126.

Seidel, K. (1976). Macrophytes and water purification. In Biological Control of Water Pollution (edited by Tourbier J. and R. W. Pierson Jr), pp. 109-112. University of Pennsylvania Press, Philadelphia, Pa.

Singh, S. S., S. P. Zhou, X. Petrie, J. R. Blackburn, S. I. Mansour and R. A. Azevedo (2005). *Functional Plant Biology*, **32**.

Shah, A. M., M. S. Memon, A. N. Memon, A. W. Ansari and B. A. Arain (2010). Analysis of Protein by Spectrophotometric and Computer Colour Based Intensity Method from Stem of Pea (*Pisum sativum*) at Different Stages, **11(2)** : 63–71.

Sharma, P., A. B. Jha, R. S. Dubey and M. Pessarakli (2012). Reactive Oxygen Species, Oxidative Damage and Antioxidative Defense Mechanism in Plants under Stressful Conditions, 2012. https://doi.org/10.1155/2012/217037.

Solano, M. L., P. Soriano and M. P. Ciria (2004). Constructed wetlands as a sustainable solution for wastewater treatment in small villages. *Biosystems Engineering*, **87(1)** : 109–118.

Smirnoff, N. (2005). *Antioxidants and reactive oxygen species in plants*. 302 pp. Oxford Blackwell Publishing Ltd. x00A3;99.95

Speck-Planche, A., V. V. Kleandrova, F. Luan and M. N. Cordeiro (2012). Predicting multiple ecotoxicological profiles in agrochemical fungicides: a multi-species chemoinformatic approach. *Ecotoxicol Environ Saf*., **80** : 308-813.

Sudhakar, Srivastava and Manoj Shrivastava (2017). Zinc supplementation imparts tolerance to arsenite stress in *Hydrilla verticillata* (L.f.) Royle. *International Journal of Phytoremediation*, **19**:4, 353-359, DOI: 10.1080/15226514.2016.1225288

Yuan, Y. A., C. B. Chen, Z. B. Chuangmu, W. A. Xiaoli, Y. A. Guiling, W. A. Qiang and Z. Zhiheng (2014). Residue of chlorpyrifos and cypermethrin in vegetables and probabilistic exposure assessment for consumers in Zhejiang Province, China. *Food Control*, **36** : 63e68.

Zhang, C., R. Hu, J. Huang, X. Huang, G. Shi and Y. Li (2016). Health effect of agricultural pesticide use in China/ : implications for the development of GM crops. Nature Publishing Group, (October), 1–8. https://doi.org/10.1038/srep34918.

Zhong, G., Z. Wu, J. Yinand L. Chai (2018). Responses of *Hydrilla verticillata* (L.f.) Royle and *Vallisneria natans* (Lour.) Hara to glyphosate exposure. *Chemosphere*, **193** : 385–393. https://doi.org/10.1016/j.chemosphere.2017.10.173.