International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.8, pp 355-365, 2016

Effect of pesticide Glyphosate on some biochemical features in cyanophyta algae *Oscillatoria limnetica*

Jasim M. Salman[1]*, Esraa Abdul-Adel[1],Ayad F.AlKaim[2]

[1]Department of Biology, College of Science, Babylon University,Iraq.
[2]Department of Chemistry, College of Science for Women, Babylon University, Iraq

Abstract : The blue green alga *Oscillatoria limnetica* was isolated from lotic ecosystem. The isolated microalga species was cultivated in BG-11 media for biomass production and to test the effect of organophosphorus glyphosate on carbohydrate, total protein, shikimik acid, flavonoid and superoxide dismutase enzyme.

Adversely depleting of the cellular activities of *O. limnetica* causing by glyphosate treatments, leading to a marked decrease in the carbohydrates, proteins, flavonoid and maximum reduction was 30.980mg/l, 22.39mg/l and 0.48 µg/g dry wt., respectively at 20mg/l of glyphosate.The shikimik acid pathway was inhibited by glyphosate, leading to an accumulation of shikimic acid. The shikimic acid content increased and the highest content was 1.38mg/l at 20mg/l. Also, superoxide dismutase enzyme activity increased along with increasing glyphosate concentrations and maximum activity was 3.14 units/ml in 20mg/l.

Keywords: pesticide ,glyphosate ,cynophytae ,*Oscillatoria limintica*, total protein ,shikmik acide ,SOD, flavonoid.

Introduction

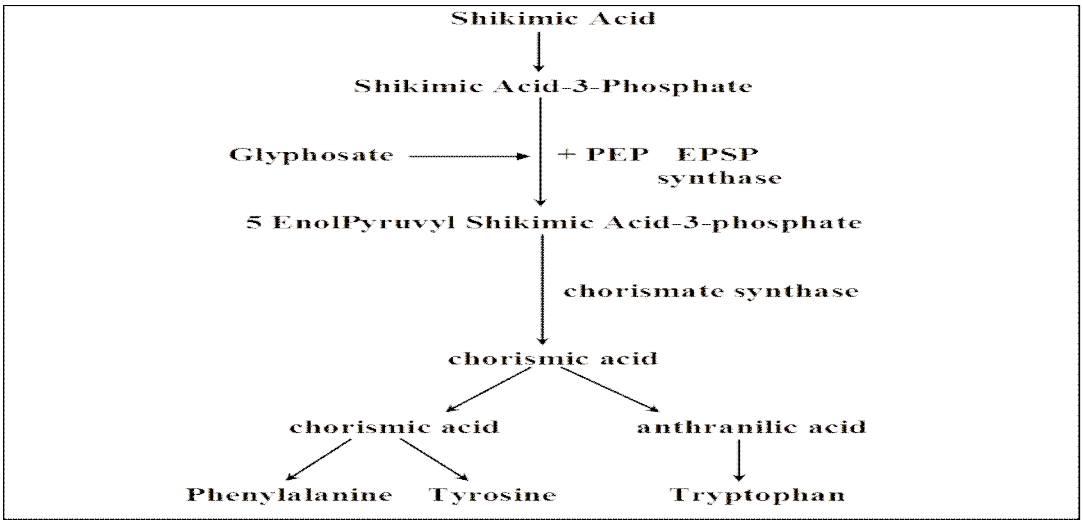
Large amounts of pesticides enter aquatic ecosystems as a result of agriculture. Adverse effects of pesticides on non-target plants are of particular concern because of the annual, widespread, and increasingly worldwide use of these chemicals[1]. Algae are essential components of aquatic ecosystems. They produce oxygen and organic substances on which most other life forms depend by providing food for other organisms, including fish and invertebrates. Toxic chemical effects on algae can directly affect the structure and function of an ecosystem, resulting in oxygen depletion, and decreased primary productivity [2]. Pesticides can affect the structure and function of aquatic communities through changing species composition of an algal community[3].

Among pesticides, glyphosate-based herbicides (GBHs), such as Roundup (R), are the most frequently used worldwide, and their residues are common contaminants of ground and surface water[4] and in food and feed[5]. This is partly due to pre-harvest desiccation treatment of non-transgenic cereals with GBHs[5], but more intensively because they are sprayed on the 80% of genetically modified plants that are engineered to tolerate GlyBH[6] and, thus, contain its residues[7].

Glyphosate is the active ingredient of more than 750 different broad-spectrum herbicides[8]. As a consequence, glyphosate jumped to a leading position among commercial pesticides from the 1970s. GlyBH use is still increasing every year[9]. Glyphosate acts on the shikimate pathway in plants through the inhibition of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme[10], which is involved in the metabolism of aromatic amino acids.

Shikimic acid is a naturally occurring organic compound, more commonly known as its anionic form shikimate, is an important intermediate in the biosynthesis of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) of plants and microorganisms wherein it operates in the biosynthesis of not just the three aromatic amino acids but also of innumerable aromatic secondary metabolites such as alkaloids, flavonoids, lignins, and aromatic antibiotics. Many of these compounds are bioactive as well as playing important roles in organism defense against biotic and abiotic stresses and environmental interactions and as such are highly physiologically important[11,12].

The biosynthesis of these essential substances are promoted by the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), the target enzyme of glyphosate. This enzyme catalyzes the transfer of the enolpyruvyl moiety of phosphoenolpyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P) to produce enolpyruvyl shikimate-3- phosphate (EPSP) and inorganic phosphate. Glyphosate resembles the transition state that transforms the reactants into products in the reaction that is catalyzed by EPSP synthase. Hence glyphosate (as a transition state analog) binds more tightly to EPSP synthase than its natural substrate and thereby prevents binding of substrate to the enzyme. This binding leads to inhibition of the enzyme and shuts down the entire pathway. Eventually this causes a deficiency in the production of the essential substances needed by the organisms to survive[13].



Scheme (1-1): The site of inhibition of glyphosate from Dill[14].

Material and Methods

Sample collection and identification

Samples of the freshwater algawere collected from artificial canal around University of Babylon in Al-Hilla city by using phytoplankton net[15]. Experimental cultures were incubated in BG-11 medium[16,17] at cool white fluorescent lamps (200) µE/m²/s with a light/dark cycle of 16/8 h. and 26± 1°C[18]. Moreover the cultures were mildly shaken by hand on alternate days[19].

Pesticide

The organophosphorus pesticide used in this study is the formulation of the herbicide glyphosate commercially available as Roundup® (containing 480 g active ingredient/L of glyphosate) was obtained from Al-Farah company, Iraq.

Experimental Design

The selected algal isolates were batch-cultured in 500 ml Erlenmeyer flasks. Into each flask 200 ml of liquid culture media, BG11 medium for *O.limnetica* ,was added. Glyphosate was added to the culture medium to the final concentrations 5 , 10 , 15 , 20 mg/l. The flasks were cultivated under the conditions described above. Response of glyphosate toxicity on *O. limnetica* (at the stationary phase) was investigated by determination of

carbohydrate, total protein, shikimik acid, flavonoid and superoxide dismutase enzyme. (All analysis done in three replicates).

Estimation of Carbohydrate

Alga samples were centrifuged by cooling centrifuge at the rate of 5000 r\min for 30 min, 4 C°. The supernatant was collected and the carbohydrate determined according to[20] method using glucose as a standard. 1ml of each sample/ standard was pipetted into a test tube, followed by addition of 1ml of 5% phenol and 5ml of 96% sulphuric acid to each tube and shaken well. After 10 minutes, the contents in the tubes were vortexed and placed in a water bath at 25-30oC for 20 minutes. The absorbance was measured at 490nm. The amount of total carbohydrate present was calculated using the standard graph prepared[21].

Estimation of Protein

After the centrifugation of alga samples by cooling centrifuge at the rate of 5000 r\min for 30 min, 4 C°, the supernatant was collected and the protein content of algae biomass was determined using the Bradford dye binding assay which is a spectroscopic analytical method used to determine the concentration of protein in a solution[22]. The Bradford assay relies on the binding of the dye Coomassie Blue G250 to protein. To protein containing extracts (100 µL) 5 ml of Bradford dye reagent was added and the contents mixed either by inversion or vortexing. The absorbance at 595nm was measured after 2min and before 1 hour. The quantity of protein was determined by interpolation from a standard curve prepared using a series of known dilution of bovine serum albumin[23].

Shikimic acid analysis

The shikimic acid concentrations (µg/ml) were evaluated following the method of [24] adapted for phytoplankton. Briefly, 50 ml of filtered growth media were resuspended in 3 ml of 0.25 M hydrochloric acid and shaken. Then, the extracts were centrifuged at 25000r/min for 15 min. The supernatant (100µl) was reacted with a 1 % solution of periodic acid. After 3 h, 1 ml of 1M sodium hydroxide and 0.6 ml of 0.1M glycine were added to the samples. Absorbance was measured at 380nm[25].

Superoxide dismutase activity assay

O.limnetica cells were collected by centrifugation at the rate of 4500 r/min for 20min, then the supernatant was decanted. The pellets were suspended in 0.9ml phosphate buffer (pH 7.4, 0.1mol/L) and then ground in an ice-bath for five min. Homogenized solution was centrifuged at 10000 r/min for 10min at 4∘C and the supernatant was used as the enzyme source[26] for SOD spectrophotometric assay.

SOD activity assay was performed by pyrogallol autoxidation method as described by[27].

Reagent:

1. Tris-buffer 50mM, pH 8.2: - this solution contains:
- Tris-base: - dissolve 0.285g of Tris-base in small amount of DW.
- EDTA: - dissolve 0.111g of EDTA in small amount of DW.
After the adjustment of pH to 8.2, the volume was made up to 100ml by DW.

2. Pyrogallol: - This solution must prepared freshly. Pyrogallol solution was prepared as described below and the material should be added sequentially. 100ml of DW. , 60µl of HCl and 0.0252g of pyrogallol.

Procedure:

	Sample	**Control**
Enzyme source	50 µl	
Tris-buffer	1 ml	1 ml
Pyrogallol	1 ml	1 ml
D.W.		50 µl
After the addition of pyrogallol, immediately read the absorbance spectrophotometrically at 420nm against blank.		

Calculation:

$$\text{SOD activity} = \frac{\%\ \text{inhibition of pyrogallol}}{50\%\ \text{inhibition from standard}}$$

$$\%\ \text{inhibition of pyrogallol autoxidation} = \frac{\Delta A\ \text{of sample}}{\Delta A\ \text{of control}} \times 100\%\ ,\ \text{where}$$

ΔA of sample = Absorbance change due to pyrogallol autoxidation in the sample reaction system

ΔAof control = Absorbance change due to pyrogallol autoxidation in the control (without cell lysate)

Determination of Total Flavonoid Content

O.limnetica cells were harvested, washed with distilled water (3 times) and used for the total flavonoid determination. The algal pellets were extracted with methanol and total flavonoid was determined by a colorimetric method as described by[28]. A 0.5 ml of each extract was made up to 1 ml with methanol. Afterwards 0.4 ml of distilled water was added, followed by 0.3 ml of 5 % $NaNO_2$ solution and the mixture was left for 5 min. Thereafter, 0.3 ml of (10%) $AlCl_3$ solution was added and allowed to stand for 6 min. Two ml of (1M) NaOH solution was added to the mixture and the final volume was adjusted to 10 ml with distilled water. The mixture was thoroughly shaken and allowed to stand for 15 min. Absorbance of the reaction mixture was read at 510nm. The concentrations of total flavonoids were determined as quercetin equivalents (mg/g of dry weight)[29].

A = 0.01069 C – 0.001163, r2=0.9998

Where A is the absorbance, C is the flavonoid concentration in µg/g of dry weight.

Statistical Analysis

General Treatment Structure was used as an experimental design. Data were analyzed to study the effect of glyphosate on *O. limnetica* and Least significant difference (LSD) was used to compare the significant difference between means at $p<0.05$.

Results and Discussion

Effect of Glyphosate on Carbohydrate Content of *O.limnetica.*

Carbohydrate content of *O.limnetica* was decreased when glyphosate concentration increased. The highest carbohydrate content inhibition 12.223% was recorded at 20mg/l and the lower carbohydrate content inhibition 1.113% was recorded at 5mg/l. Significant differences were recorded among all treatments (Fig. 1). This may be due to inhibition of photosynthesis and photochemical efficiency of photosystemII in cyanophyta that resulting from the photooxidation of chlorophyll a, the destruction of accessory pigments to levels below their protective threshold, together with impaired energy transfer to photosystemII reaction centers, as a consequence of transformational variations to phycobilisomes[30].

The finding of this study agreed with[31] who used*Anabaena cylindrica*, *Chlamydomonas reihardii*, *Chlorella vulgaris*, and *Chroococcus turgidus* to explore the physiological and biochemical effects of glyphosate on algae. The common visible symptoms of glyphosate toxicity in all algal cells were bleaching effect and reduction the contents of carbohydrate. The results highly suggested that glyphosate injured the algal cells by destruction of photosynthetic pigments and resulted in lowering the contents of carbohydrate in algal cells. Identical results were stated by[32] who suggested that there was a greater reduction in the level of carbohydrate (44%) observed in *Oscillatoria pseudogeminata* at the highest concentration (1000ppm) of carbaryl on the 24th day and the lesser reduction (14%) at 100ppm as compared to control. As the concentration of pesticide increases, the total carbohydrate level decreased considerably. Also, in previous studies, the inhibition of carbohydrate production in cyanophyta has been observed at high external concentrations of glyphosate[33,34].

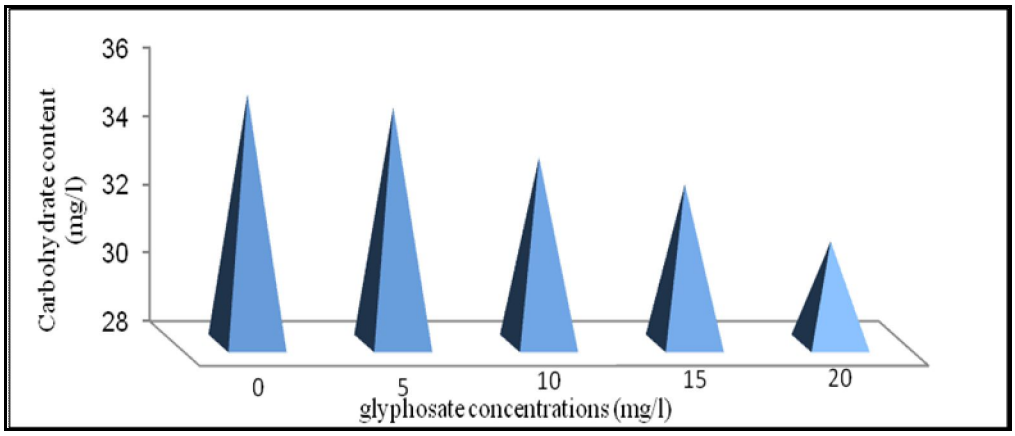


Fig. 1: Carbohydrate content of *O.limnetica* at different glyphosateconcentrations (mg/l). *(p<0.05)Significant differences between control and all treatments.

Effect of Glyphosate on Total Protein Contentof *O.limnetica*

The total protein content of *O.limnetica* was less than the control in 5, 10, 15 and 20mg/l. The highest protein content inhibition 37.45% was recorded at 20mg/l and the lower protein content inhibition 9.52% was recorded at 5mg/l.Significant differences were recorded among all treatments (Fig. 2). This may be due to inhibit the aromatic amino acid synthesis, which results in the inhibition of nucleic acid metabolism, protein synthesis and the abundance of proteins associated with photosystemII and the changes in protein synthesis in algae grown under stress could be due to changes in gene expression[35-37]. Also have observed decrease in protein may be due to the deficiency of protein synthesis or increase in the rate of its degradation of amino acids, which may be fed to tricarboxylic acid (TCA) cycle through aminotransferases probably to cope up with high energy demands in order to meet the stress condition[38].This study is in agreement with a study by[39] which reported that the soluble and insoluble proteins was decreased in *Scenedesmus* with increasing glyphosate as well as soluble proteins were decreased with increasing of glyphosate herbicide to *Merismopedia*. The same results were stated by[40] who reported that the accumulation protein decreased in *Chlamydomonas mexicana* in the presence of 15mg/l acephate and imidacloprid for 12days. Additionally[41] observed that the protein content of *Anabaena sp.* decreased beyond 2.5µg/ml of chlorpyrifos.

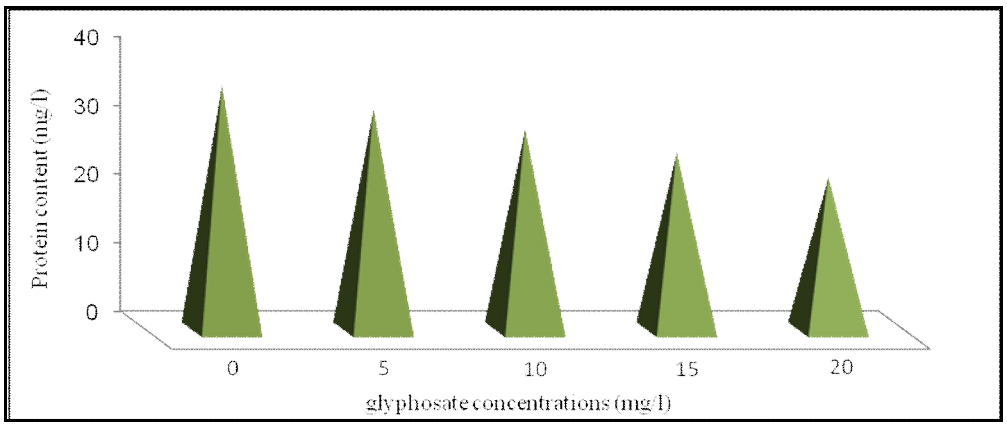


Fig. 2: Protein content of *O.limnetica* at different glyphosate concentrations (mg/l). *(p<0.05)Significant differences between control and all treatments.

Effect of Glyphosate on Shikimik acid Contentof *O.limnetica*

The shikimic acid content showed a significant increase after *O.limnetica* exposed to 5, 10, 15 and 20mg/l of glyphosate (Fig. 3). This may be due to the inhibition of shikimic acid pathway by glyphosate, by competing with the enzyme 5-enolpyruvoylshikimate3-phosphate synthease (EPSPS) and that leading to the accumulation of the substrate shikimate 3-phosphate[42-44].The result is in agreement with[45] who found that the shikimic acid content, after glyphosate exposure, showing a significant increase in phytoplankton cells exposed

to 500 and 1000 µg/l of glyphosate. This result also proves that the shikimic acid content can based as a biomarker of the effects of glyphosate-based herbicides on phytoplankton communities. Similarly, [46] found that the cells of *Synechocystis* 6803, grown in the presence of 1mM and 5mM isopropylamine salt for 96h, contained 135µg^{-1} shikimate compared to 53µg^{-1} in untreated cells.

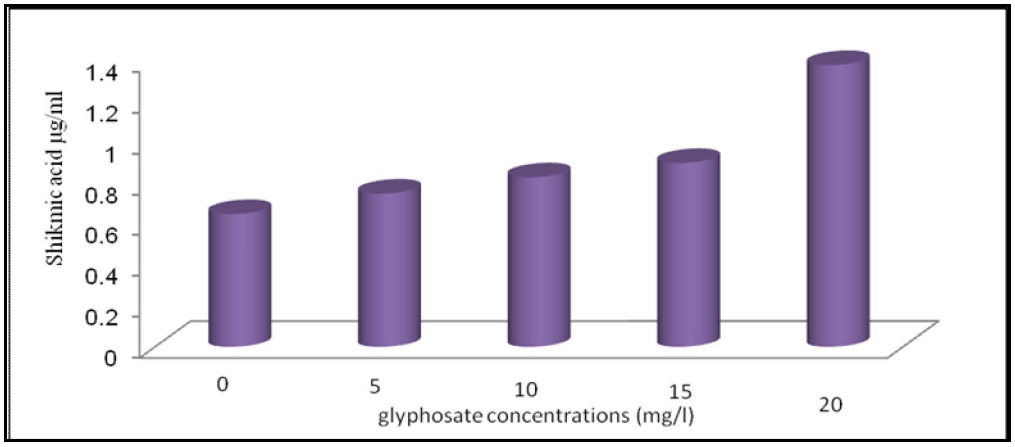


Fig. 3: Shikimik acid content of *O.limnetica* at different glyphosate concentrations (mg/l). *(p<0.05)Significant differences between control and all treatments.

Effect of Glyphosate on Superoxide Dismutase Enzyme Acivity (SOD) of *O.limnetica*

Toxicity of herbicides may lead to the generation of free radicals and cyanophyta may respond to this stress by inducing antioxidant defense. SOD enzyme in *O.limnetica* grown in glyphosate (5, 10, 15 and 20 mg/l) was increased (Fig. 4).The increased activities of SOD in *O. limnetica* indicated that glyphosate stress may have stimulated the generation of reactive oxygen species which were reduced by the elevated levels of these enzymes and helped algal cells to tolerate herbicide stress[47,48] reported that the action of glyphosate formulations may be involved in increasing the production of superoxide anions and therefore in triggering oxidative stress and antioxidant responses. In compatible with this result[49], studied the stress responses of *Anabaena cylindrical* for its to sublethal concentrations (0.75-2mM) of bentazon. They found that the activities of antioxidant enzymes such as superoxide dismutase (SOD) increased in a time and herbicide dose-response manner were higher than those in the control samples after 72 h. Other herbicides such as glufosinate[50] and paraquat[51] , increased SOD activities at 0.5 by 3-4 times over control cultures in the unicellular green alga *Chlorella vulgaris*. Similar results were stated by[52] who found that enzymatic defences increased when glyphosate was present in the growth medium of *Chlorella kessleri*. Also,the enzyme SOD was stimulated significantly with increasing concentration of malathion as 18%, 38%, 53% and 93% in 25, 50, 75 and 100 µg/ml malathion respectively compared to untreated control[53].

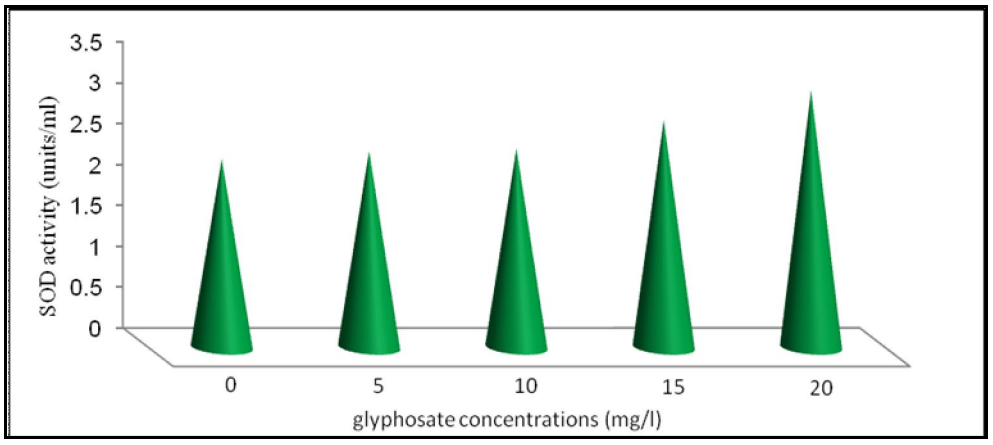


Fig. 4: SOD activity of *O.limnetica* at different glyphosate concentrations (mg/l). *(p<0.05)Significant differences between control and all treatments.

Effect of Glyphosate on Flavonoid Content of *O.limnetica*

Flavonoid content of *O.limnetica* was decreased when glyphosate concentration increased. Significant differences were recorded among all treatments (Fig. 5). This may be due to the blockage of shikimik acid pathway which leading to a deficiency of significant end products such as lignins, alkaloids, and flavonoid and a decrease in CO_2 fixation and biomass production in a dose dependent manner[54] .The finding of this study agreed with[55] who observed suppressing of the biosynthesis of lipid, protein and flavonoids by different pesticides in different physiological ways[56-76].

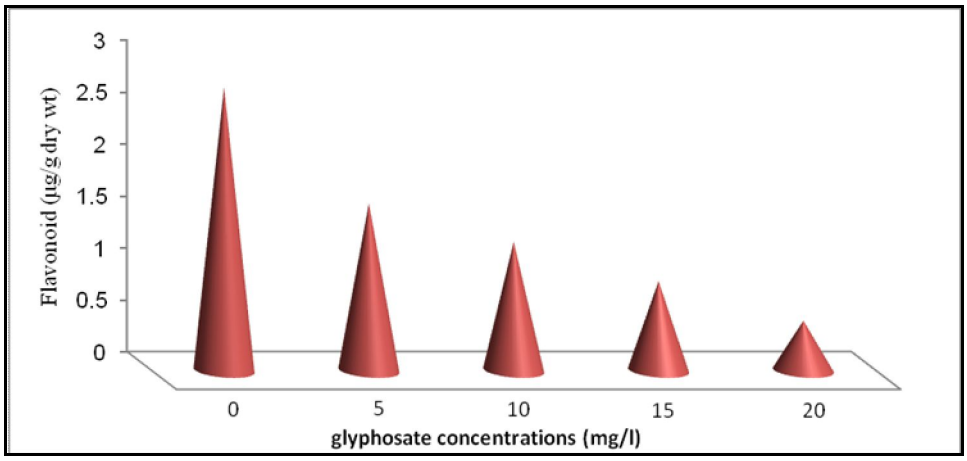


Fig. 5: Flavonoid content of *O.limnetica* at different glyphosate concentrations (mg/l).
***(p<0.05)Significant differences between control and all treatments.**

Conclusions

Glyphosate treatments affected the activities in the cyanophyta species by reducing the carbohydrate, total protein, flavonoid as well as increased shikimik acid and superoxide dismutase enzyme activity.

Acknowledgment

We thank the department of Biology,College of Science and Environmental Research Center,University of Babylon for material supported.

References

1. Vander Brink P.J. and Ter Braak, C.J.F. 1999, Principal response curves: analysis of time-dependent multivariate responses of biological community to stress. Environ. Toxicol. Chem. 18(2): 138-148.
2. Cetin, A. K. and Mert, N. Growth, 2006, Rate of Scenedesmus acutus (Meyen) in cultures exposed to trifluralin, Pol. J. Environ. Stud., 15(4) : 631-633.
3. El Jay, A. 1996, Toxic effect of organic solvents on the growth of Chlorella vulgaris and Selenastrum capricornutum. Bull. Environ. Contam. Toxicol., 57 (2) : 191-198.
4. ANSES. Evaluation of the Risks of Pesticide Residues in Water Distribution. 2016.
5. Székács, A. and Darvas, B. Forty years with glyphosate. In Herbicides—Properties, Synthesis and Control of Weeds; In: Hasaneen, M.N. [Ed].; Elsevier: Burlington, MA, USA, 2012.
6. James, C. Global Status of Commercialized Biotech/GM Crops; ISAAA Brief 49 , 2014.
7. Bøhn, T.; Cuhra, M.; Traavik, T.; Sanden, M.; Fagan, J. and Primicerio, R. 2014, Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans. Food Chemistry, 153 : 207–215.
8. Guyton, K.Z.; Loomis, D.; Grosse, Y.; El Ghissassi, F.; Benbrahim-Tallaa, L.; Guha, N.; Scoccianti, C.; Mattock, H. and Straif, K., 2015, Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. International Agency for Research on Cancer Monograph Working Group, I.L.F, Lancet Oncol, 16 (5) : 490 - 491.

9. Benbrook, C.M. 2012, Impacts of genetically engineered crops on pesticide use in the U.S. the first sixteen years. Environmental Sciences Europe, 24 : 24.
10. Boocock, M.R. and Coggins, J.R. 1983, Kinetics of 5-enolpyruvylshikimate-3-phosphate synthase inhibition by glyphosate. FEBS Lett, 154 (1) : pp.127-133.
11. Herrmann, K.M. and Weaver, L.M. 1999, The Shikimate Pathway. Annual Review of Plant Physiology and Plant Molecular Biology, 50: 473-503.
12. Maeda, H. and Dudareva, N. 2012, The shikimate pathway and aromatic amino acid biosynthesis in plants. Annual Review of Plant Biology, 63:73-105.
13. Pollegioni, L.; Schonbrunn, E. and Siehl, D. 2011, Molecular basis of glyphosate resistance-different approaches through protein engineering. FEBS Journal, 278 (16): 2753-2766.
14. Dill, G. M. Glyphosate resistant crops: history, status and future. Pest Management Science, 61: 219-224. 2005.
15. Salman, J.M. and Abdul-Adel, E. 2015, Potential use of cyanophyta species Oscillatoria limnetica in bioremediation of organophosphorus herbicide glyphosate. Mesopotamia Environmental Journal, 1 (4),pp.15-26.
16. Andersen, R.A. and Kawachi, M. Traditional Microalgae Isolation Techniques. In: Andersen, R.A. [Ed.] Algal Culturing Techniques. Academic Press, San Diego, pp. 578.2005.
17. Rippka, R.; Deruelles, J.; Waterbury, J.B.; Herdman, M. and Stanier, R.Y. 1979, Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology, 111 (1): 1-61.
18. Shoker R. M. H. and Jawad, A. M. .2013, Evaluation of Isolated Compounds Activity from Convolvulus Arvensis Against Algae. Iraqi Journal of Science, 54 (1),pp.62-71.
19. Selvan, B. K.; Revathi, M.; Piriya, P. S. and Vasan, P. T. 2013, Biodiesel production from marine cyanobacteria cultured in plate and tubular photobioreactors. Indian Journal of expermnetal biology, Vol.51,No.3,pp.262-268..
20. Dubois, M.; Gilles, K.A.; Hamiton, J.K.; Robers, P.A. and Smith, F. 1956, Colorimetric method for determination of sugar and substances. Anal. Chem., 28 (3): 350-356.
21. Nordin, N.; Yusof, N. and Samsudin, S. 2014, Microalgae Biomass Production And Nitrate Removal From Landfill Leachate. Proceeding of International Conference On Research, Implementation And Education Of Mathematics And Sciences, 73-82.
22. Bradford, M.M. 1976, A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding. Analytical Biochemistry, 72 (1-2): 248-254.
23. Oshungboye, K.T. Microalgae biomass as fermentation feedstock. M.Sc. Thesis submitted to Environmental Biotechnology, University of Rhodes, South Africa. 2011.
24. Singh, B.K.; and Shaner, D.L. Rapid Determination of Glyphosate Injury to Plants and Identification of Glyphosate-Resistant Plants. Weed Technology, 12 (3): 527-530. 1998.
25. Smedbol, É.; Gomes, M.P.; Paquet, S.; Laurent Lepage, M.L.; Lucotte, M. and Juneau, P. Low Concentrations Of Glyphosate-Based Herbicide Have Deleterious Effects On A Freshwater Phytoplankton Community From An Agricultural Stream. The scientific journal of aquatic toxicology, 40-81.2013.
26. Jia, W.; Huang, X. and Li, C. A Preliminary Study of the Algicidal Mechanism of Bioactive Metabolites of Brevibacillus laterosporus on Oscillatoria in Prawn Ponds. The Scientific World Journal, 1-11. 2014.
27. Marklund, S. and Marklund, G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry, 47 (3): 469–474. 1974.
28. Zhishen, J.; Mengcheng, T. and Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64 (4): 555-559. 1999.
29. Abd El-Aty, A.M.; Mohamed, A.A. and Samhanl, F.A. In vitro antioxidant and antibacterial activities of two fresh water Cyanobacterial species, Oscillatoria agardhii and Anabaena sphaerica. Journal of Applied Pharmaceutical Science, 4 (7): 69-75. 2014.
30. Singh, S. and Shikha, D. P. Influence of glyphosate on photosynthetic properties of wild type and mutant strains of cyanobacterium Anabaena doliolum. Current Science, 86 (4): 571-576. 2004.
31. Baolong and Tsai . The fate of glyphosate in water hyacinth and its physiological and biochemical influences on growth of algae. Ph.D Thesis submitted to North Carolina State Univ; Raleigh, NC (USA); University Microfilms, pp. 140. 1989.

32. Bakiyaraj, R. ; Baskaran, L. and Senthilkumar, T. Isolation and identification of cynobacteria (Oscillatoria pseudogeminataG.schmid) from marine water and its potential on remediation of pesticide. International journal of current microbiology and applied sciences, 3 (3): 256-267. 2014.
33. Mansour, F.A. ; Soliman, A.I. ; Shaaban-Dessouki, S.A. and Hussein, M.H. Effect of herbicides on cyanobacteria. I . changes in carbohydrate content, PMase and GOT activities in Nostoc kihlrani and Anabaena oscillarioides. Phykos, 33: 153-162. 1994.
34. Kannan, V.; Srinivasan, S.; Kathiravan, G.; Raju, K. ; Sbramanian, K. and Sivakumar, D. Influence of glyphosate on Mastigocladus laminosus. Journal of Environmental Biology, 20: 199-206. 1999.
35. Tiwari, D. N. ; Pandey, A. K. ; Mishra, A. K. and Srivastava, V. Role of 2,4-D on growth and macromolecular synthesis in a cyanobacterium Anabaena cylindrica. Indian Journal of Botany, 5 (2): 111-114. 1982.
36. Duke, S.O. Glyphosate. In: Kearney, P.C. and Kaufman, D.D. 1988. (Ed.). Herbicides: chemistry, degradation and mode of action. Marcel Dekker, New York, NY., 3:1-70.
37. Vivancos, P.D. ; Driscoll, S.P. ; Bulman, C.A. ; Ying, L. ; Emami, K. ; Treumann, A. ; Mauve, C. ; Noctor, G. and Foyer, C.H. 2011.Perturbations of amino acid metabolism associated with glyphosate-dependent inhibition of shikimic acid metabolism affect cellular redox homeostasis and alter the abundance of proteins involved in photosynthesis and photorespiration. Plant Physiology, 157 (1): 256–268.
38. Singh, S. and Bhati, D.P.S. 1994.Evaluation of liver protein due to stress under 2, 4-D. intoxication in Channa punctatus (Bloch) Bull. Environ. Contam. Toxicol., 53: 149-152.
39. Issa, A.A.E. ; Adam, M.S. and Fawzy, M.A. 2013.Alterations in some metabolic activities of Scenedesmus quadricauda and Merismopedia glauca in response to glyphosate herbicide. Journal of Biology and Earth Sciences, 3 (1): 17-23.
40. Kumar, M.S. ; Kabra, A.N. ; Min, B. ; El-Dalatony, M.M. ; Xiong, J.; Thajuddin, N. ; Lee, D.S. and Jeon, B. H. 2015.Insecticides induced biochemical changes in freshwater microalga Chlamydomonas Mexicana. Environmental Science and Pollution Research, 1-9.
41. Deviram, G.V. and Prasuna, R.G. 2012.Effect of Chlorpyriphos on Cyanobacterial Isolates from Rice Fields of Coastal Areas. International Journal of Mathematics Research, 4 (4): 377-386.
42. Dewick, P.M. 1998.The biosynthesis of shikimate metabolites. Natural product reports, 11: 173-203.
43. Fedtke, C. and Duke, SO. 2005. Herbicides. In: Hock, B. and Elstner, EF. [Eds.]. Plant toxicology. New York: Marcel Dekker, pp. 247–330.
44. Duke, S.O. and Powles, S.B. 2008.Glyphosate: A once-in-a century herbicide. Pest Managment Science, 64 (4): 319-25.
45. Smedbol, É.; Gomes, M.P.; Paquet, S.; Laurent Lepage, M.L.; Lucotte, M. and Juneau, P.2013.Low Concentrations Of Glyphosate-Based Herbicide Have Deleterious Effects On A Freshwater Phytoplankton Community From An Agricultural Stream. The scientific journal of aquatic toxicology, 40-81.
46. Powell, H.A.N.; Kerby, W. and Rowell, P. 1991.Natural tolerance of cyanobacteria to the herbicide glyphosate. New Phytologist, 119 (3): 421-426.
47. Bagchi, D. ; Bagchi, M. ; Hassoun, E.A. and Stohs, S.J. 1995, In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. Toxicology, 104 (1-3): 129-140.
48. Peixoto, F. 2005, Comparative effects of the Roundup and glyphosate on mitochon-drial oxidative phosphorylation. Chemosphere, 61:115–1122.
49. Galhano, V. ; Peixoto, F. and Gomes-Laranjo, J. 2010, Bentazon triggers the promotion of oxidative damage in the Portuguese ricefield cyanobacterium Anabaena cylindrica: response of the antioxidant system. Environmental Toxicology, 25 (5): 517-526.
50. Qian, H.; Chen, W.; Sheng, G.D.; Xu, X.; Liu, W. and Fu, Z. 2008, Effects of glufosinate on antioxidant enzymes, subcellular structure and gene expression in unicellular green alga Chlorella vulgaris. Aquatic Toxicology, 88 (4): 301–307.
51. Qian, H. ; Chen, W. ; Sun, L. ; Jin, Y. ; Liu, W. and Fu, Z. 2009, Inhibitory effect of paraquat on photosynthesis and the response to oxidative stress in Chlorella vulgaris. Ecotoxicology, 18 (5): 537–543.
52. Romero, D.M. ; Molina, M.C.R.D and Jua´rez, A´.B. 2011, Oxidative stress induced by a commercial glyphosate formulation in a tolerant strain of Chlorella kessleri. Ecotoxicology and Environmental Safety, 74: 741-747.

53. Manikar, N. ; Kumar, S. ; Habib, K. and Fatma, T. 2013, Biochemical Analysis of Anabaena variabilis Exposed to Malathion Pesticide with Special Reference to Oxidative Stress and Osmolytes. International Journal of Innovative Research in Science, Engineering and Technology, 2 (10): 5403-5420.
54. Olesen, C.F. and Cedergreen, N. 2010, Glyphosate uncouples gas exchange and chlorophyll fluorescence. Pest Management Science, 66 (5): 536-542.
55. Fairchild, F.J.; Ruessler, S.D. and Carlson, R.A. 1998, Comparative Sensitivty of Five Species of Macrophytes and Six Species of Algae to Atrazine, Metribuzin, Alachlor, and Metochlor. Environmental Toxicology and Chemistry, 17 (9): 1830-1834.
56. Al-Hamairy , A , K. 2016, Effect of lipopolysaccharide extracted from escherichia coli on the growth and development of promastigote of leishmania tropica (in vitro study), International Journal of PharmTech Research,9(4), 377-386.
57. Al-Gubury H. Y., Fairooz N. Y., Aljeboree A. M., Alqaraguly M. B., and Alkaim A. F.2015, Photcatalytic degradation n-undecane using coupled ZnO-Co2O3, Int. J. Chem. Sci., 13(2): p. 863-874.
58. Alqaragully M.B., AL-Gubury H. Y, Aljeboree A.M., Karam F.F., and Alkaim A. F. 2015, Monoethanolamine: Production plant, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 6(5): p. 1287-1296.
59. Abid, F. M.; Salman, J. M., 2012. Liquid chromatographic method for determination of biogenic amines in imported fish and meat products, asian journal of chemistry, 24: 5763-5765.
60. Algubili A. M., Alrobayi E. M., and Alkaim A. F.,2015, Photocatalytic degradation of remazol brilliant blue dye by ZnO/UV process. Int. J. Chem. Sci., 13(2): p. 911-921.
61. Aljeboree, A. M.,2016, Adsorption of crystal violet dye by Fugas Sawdust from aqueous solution. International Journal of ChemTech Research, 9(3): p. 412-423.
62. Aljeboree, A. M.; Radi, N.; Ahmed, Z.; Alkaim, A. F., 2014.The use of sawdust as by product adsorbent of organic pollutant from wastewater: Adsorption of maxilon blue dye, International Journal of chemical sciences, 12: 1239-1252.
63. Omran, A. R.; Baiee, M. A.; Juda, S. A.; Salman, J. M.; Alkaim, A. F., 2016.Removal of congo red dye from aqueous solution using a new adsorbent surface developed from aquatic plant (Phragmites australis), of ChemTech Research, 9: 334-342.
64. Salman, J. M.; Al-Mamoori, A. M. J.; Al-Saadi, A. H.; al Terehi, M. N., 2012.Dna damage in common carp fish (cyprenus carpio l.) induced by acute exposure to copper & cadmium, World Applied Sciences Journal, 20: 679-682.
65. Jafar, N. N.; Al-Dahmoshi, H. O. M.; Jeburalmamoori, A. M.; Al-Khafajii, N. S. K.; Al-Masoudi, N., 2013.Synthesis and biological activity of new derivatives of 6-chloro-5-((4-chlorophenyl) diazenyl) pyrimidine-2,4-diamine and 4-chloro-6-methoxy-N,N-dimethylpyrimidin-2-amine, Biomedical and Pharmacology Journal, 6: 453-465.
66. Alkaim, A. F.; Hussein, F. H., 2012. Photocatalytic degradation of EDTA by using TiO2 suspension, International Journal of Chemical Sciences, 10: 586-598.
67. Hashim, F. S.; Almaamori, M. H.; Hamood, F. J., 2016.Effect of silica on the mechanical properties of rubber reclaim composite, International Journal of ChemTech Research, 9: 325-333.
68. Al-Terehi, M., Al-Saadi, A. H., Al-Sherefi, A. N., and Zaidan, H. K., 2016.Optimization polyplexe stability in different glucose concentrations, International Journal of ChemTech Research, 9: 396-401.
69. Ai-Mashhedy, L. A. M.; Fijer, A. N., 2016.Acute toxicity of food additives tartrazine and carmoisine on white male mice, International Journal of PharmTech Research, 9: 364-367.
70. Raheem, H. Q.; Al-Thahab, A. A.; Abd, F. G., 2016. Different methods for detection sliver nanoparticles produced by proteus mirabilis bacteria, International Journal of PharmTech Research, 9: 368-376.
71. Abdulrazzak, F. H., 2016.Enhance photocatalytic activity of TiO2 by carbon nanotubes, International Journal of ChemTech Research, 9: 431-443.
72. Hadi, A. G., 2016.Removal of Fe (II) and Zn (II) ions from aqueous solutions by synthesized chitosan, International Journal of ChemTech Research, 9: 343-349.
73. Al-Gazally, M. E.; Al-Awad, A. S.; Kzar, H. H., 2016.Assessment of antioxidant status in different genotypes/phenotypes at codon 72 of TP53 gene for patients with sporadic colorectal cancer in Babylon province, International Journal of PharmTech Research, 9: 280-286.

74. Karam, F. F.; Hussein, F. H.; Baqir, S. J.; Alkaim, A. F., 2016. Optimal conditions for treatment of contaminated waters with anthracene by Fenton processes in close system reactor, Journal of Chemical and Pharmaceutical Science, 9: 1111-1115.
75. Kamil, A. M.; Mohammed, H. T.; Alkaim, A. F.; Hussein, F. H., 2016. Adsorption of Congo red on multiwall carbon nanotubes: Effect of operational parameters, Journal of Chemical and Pharmaceutical Sciences, 9: 1128-1133.
76. Raheem, R. A.; Al-gubury, H. Y.; Aljeboree, A. M.; Alkaim, A. F., 2016. Photocatalytic degradation of reactive green dye by using Zinc oxide, journal of Chemical and Pharmaceutical Science, 9: 1134-1138.

*****