1st Int. Symp. Phycol. (ISP).
(21-22) Oct. 2000.

Egypt. J. Phycol. Vol. 1, 2000

EFFECT OF GLYPHOSATE HERBICIDE ON GROWTH, PHOTOSYNTHESIS AND SOME METABOLIC ACTIVITIES OF THE GREEN ALGA *CHLORELLA KESSLERI* (CHLOROPHYTA)

Mostafa M. EL-Sheekh

Botany Department, Faculty of Science, Tanta University, 31527 Tanta, Egypt.
Email: msheekh@dec1.tanta.eun.eg Fax: 20-40-3350804

Abstract

The toxic effects of glyphosate herbicide on growth, photosynthesis, chlorophyll and amino acids contents by the fresh water green alga *Chlorella kessleri*, were determined. The lower concentrations 5 and 10 mM were not severely toxic to the alga. However, the higher concentrations 15 and 20 mM exerted more toxicity effects. Algal growth, photosynthetic activity and chlorophyll contents were not affected severely by low concentrations, while high concentrations (20 mM), inhibited growth parameters by about 50%. The addition of the aromatic amino acids, phenyl alanine, tryptophane and tyrosine to the growth medium, recovered the growth by about 34% during the incubation period. The amino acids content of the control, 20 mM-treated and 20 mM-treated in addition to the aromatic amino acids were estimated and compared. The results also showed that the addition of the aromatic amino acids to the alga treated with 20 mM glyphosate, recovered the biosynthesis of the aromatic amino acids and also the other amino acids.

Key words: algae, glyphosate herbicide, growth, protein, photosynthesis, amino acids.

Introduction

Glyphosate [N-phosphonomethyl glycine] is a broad spectrum herbicide and the most common used herbicide. Its action is mediated by inhibiting one of the key enzymes of aromatic amino acid biosynthesis 5-enolpyruvyl shikimate -3- phosphate synthase (Jaworski, 1972). This nonselective, broad spectrum, postmergence herbicide is registered for use in more than 50 crops and is used extensively for vegetation control and management in many nonagricultural settings. Atkinson (1985) reviewed the toxicological properties of glyphosate. Glyphosate does not move readily through soil because of strong complexing properties, therefore it is unlikely to enter the aquatic environment in significant amounts unless for aquatic weed control (Bowmer, 1982; Barrett, 1985) or unless it washes from foliage into streams or canals (Newton *et al.* 1994). Glyphosate can move considerable distances in canal or stream waters. Come *et al.* (1976) accounted for 58% of applied glyphosate at distance 8 and 14.4 Km downstream from sites of introduction into the canals. He showed also that approximately 30% of the glyphosate were lost in the first 1.6 Km, presumably due to adsorption to suspended material that settled out. Glyphosate may enter into aquatic systems from the treated terrestrial land through surface runoff movements, spray drift, or direct overspray applications. Similarities between the physiology of higher plants and phytoplankton in aquatic environments surely will cause adverse effects on the latter non-target primary producers. Phytoplanktons in streams and ponds form an integral part of the aquatic food web providing food for larger organisms such as zooplankton and fish. Several studies indicate

(ISSN: 1110-8649)

that this herbicide is toxic for aquatic flora and fauna (Becerril *et al.* 1989; Peterson *et al.* 1994; Gardner *et al.*1997; Roberts *et al.* 1998; Wong, 2000). Glyphosate inhibits the activity of 5-enolpyruvyl shikimate -3- phosphate synthase[(EPSP) synthase], an enzyme of the shikimic acid pathway in a wide variety of plants and organisms (Duke, 1988). Glyphosate inhibits other enzymes of the shikimic acid pathway to a lesser degree than EPSP synthase. The blockage of the shikimic acid pathway leads to a depletion of the free pool of aromatic acids (Hoagland *et al.* 1978; Duke and Hoagland, 1985). Furthermore, glyphosate greatly lessens increases in phenylalanine levels caused by inhibitors of the enzyme phenylalanine ammonia-lyase (Duke *et al.* 1980; Laber *et al.*1986).

The effects of glyphosate on protein synthesis are poorly documented. Cole *et al.* (1980) found that radiolabeled leucine incorporation into buds of quack grass was inhibited by glyphosate, however, incorporation of [^{14}C] phenylalanine was affected to a much lesser extent. The data indicated that the inhibition of protein synthesis by glyphosate was due to depletion of the aromatic amino acids pool rather than to direct or indirect interference with or damage to the protein synthesizing process. The genus *Chlorella* is very common in all kinds of freshwaters. The paucity of published research on the toxicity of glyphosate towards freshwater algae in general and *Chlorella* in particular is the consequence of the general lack of toxicity of glyphosate component to most other pesticides (El-Sheekh *et al.* 1994).

The aim of this work is to assess the possible alterations in growth and some physiological processes in response to glyphosate herbicide in the freshwater *Chlorella kessleri* which represent the main phytoplankton of any water body which receive the herbicides.

Materials and Methods

Organisms and growth conditions: The green alga *Chlorella kessleri,* obtained from the algal culture collection, Plant Physiology Institute, University of Götingen, Germany, was cultured in tris-acetate-phosphate medium (TAP medium), (Harris, 1989). The cultures were illuminated continuously with fluorescent tubes maintaining the desired light intensity (5000 lux), incubated at 25 °C and shaken on a rotatory shaker incubator.

Growth parameters measurements: Growth of the cultures was monitored either by measuring the optical density of the cell suspension spectrophotometrically at 560 nm (Wetherell, 1961) or as the increase in dry weight production as reported by Leganes et al., (1987). For determination of the dry weight, 10 ml were removed from the culture and centrifuged at 5000 rpm for 10 min. The pellet washed twice with dist. water and incubated for 24 hours at 95 °C, till constant weight.

Chlorophyll determination: Chlorophyll a and b content was determined according to Lichtenthaler and Wellburn (1983). In this method a known volume of the algae culture was centrifuged at 3000 rpm for 15 min and the pellet was extracted by 100% dry methanol for 15 min at 55 °C. The absorbance of the supernatant was read at 653 and 666 nm in spectrophotometer against methanol blank.

Measurement of photosynthetic activity: Oxygen evolution or uptake (dark respiration) measurements were done with a Clark-type oxygen electrode in 3 ml samples in a thermostated closed prespex cuvette at 25°C at saturated light. Electrode was calibrated to steady state of oxygen in water at 25°C (220 µmol ml^{-1}), zero level was estimated by bubbling water and with nitrogen gas. Data were recorded by chart recorder.

Estimation of total soluble proteins: Protein was estimated by the method of Bradford (1976) using bovine serum albumin as a standard at 595 nm.

Quantitative estimation of total amino acids: The protocol used by Moore et al., (1958) was followed to estimate the total amino acids. According to this method, 0.05 gm of each dried algal sample was mixed with 10 ml of 6N HCl containing traces of mercaptoethanol (5µl/10 ml acid). The samples were hydrolyzed at 110 °C for 24 hours. The hydrolyzed sample was diluted at 25 ml with dist. water and dried by slow evacuation. The dried residue was dissolved in 0.2M sodium citrate buffer (pH 2.2) and run in Beckman amino acid analyzer.

Results and Discussion

Effect of glyphosate on growth of *Chlorella kessleri*

Chlorella kessleri was able to grow fast in acetate containing medium mixotrophically (EL-Sheekh, 1999). The rate of growth of the alga in this medium was faster than other nutrient media (data not shown). The assessment of the toxic effect of glyphosate on *C. kessleri* indicated a decrease in growth, with respect to the control in the cultures exposed to all applied concentrations after 72 or 96 hours. The growth of the alga was affected by the application of the different concentrations of the glyphosate herbicide as shown in Fig. 1. It is evident that by increasing the concentration of the herbicide there was a decrease in growth as measured by optical density. The inhibition was maximum and accounted for about 54% of control at the concentration 20mM after 96 hours. The inhibitory effect was also increased by increasing the incubation period. The growth of the alga was also assessed by measuring the increase in the dry weight after the incubation period (96 hours). It was found that results of the dry weight is in accordance with the results of optical density i.e. the inhibition increased by increasing the concentration of herbicide and it reached maximum inhibition 52% at the concentration 20 mM (Fig. 2). Saenz *et al.* (1997) indicated that the two strains of chlorococcal algae *Scenedesmus acutus* and *Scenedesmus quadricauda* responded differently in growth to the application of different concentrations of glyphosate herbicide. However, they concluded that the low concentrations of the herbicide did not significantly affect the growth, while the high concentration 40 mg Gly/L showed a notable inhibition of growth at 96 hours of exposure. *Scenedesmus acutus* growth was completely inhibited by 20 mg Gly/L. Wong (2000) also indicated that the growth of *Scenedesmus quadricauda* Berb 614 was significantly inhibited by 2 mg/L and completely inhibited by 20 mg/L. From the above results and the discussed results of Saenz *et al.* (1997) and Wong (2000), it could be concluded that algae respond differently to different concentrations of the glyphosate herbicide and also the toxicity of the herbicide is species dependent. In this connection Peterson *et al.* (1994) indicated that the green algae *Scenedesmus quadricauda, Selenastrum capricornutum*, the

diatoms *Nitzschia* sp., *Cyclotella meneghiniana* and five cyanobacteria, responded differently to glyphosate in that the green algae were least sensitive, but the diatoms and one cyanobacterium were the only organisms that showed sensitivity to glyphosate. In the present work a lower concentrations of glyphosate were used for *Chlorella* than that used by Saenz *et al.* (1997) and Wong (2000) to *Scenedesmus*. The data rose in comparison with the possible glyphosate concentration that it could be found in the aquatic environment, whereas the recommended application rates for terrestrial weed control (0.25-2kg Gly a.i./Ha). If 1-3% of the application dose may be recovered in aquatic environments, so the possible glyphosate concentrations found would be 0.7 to 4.8 mg Gly/L (Alberdi *et al.* 1996).

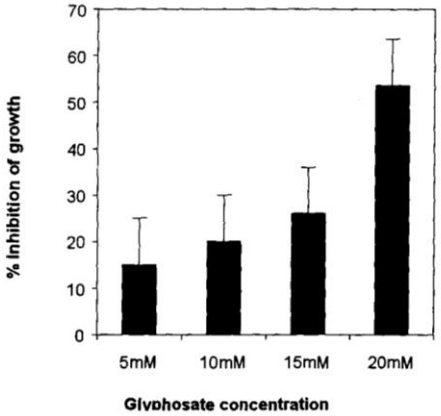

Fig. 1. Effect of different concentrations of glyphosate herbicide on growth of *Chlorella kessleri* (expressed as percentage of control culture). Error bars represent the standard error of the mean of three replicates.

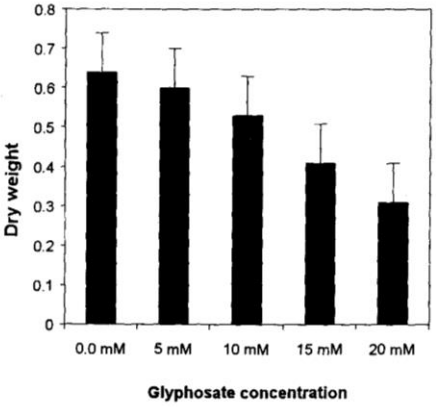

Fig. 2. Effect of different concentrations of glyphosate herbicide on dry Weight production of *Chlorella kessleri*. Error bars represent the standard error of the mean of three replicates.

Effect of Glyphosate on Chlorophyll content

It is documented that glyphosate active ingredient has relatively strong effect on chlorophyll synthesis. Glyphosate inhibits the synthesis of the chlorophyll precursor 5-aminolevulinic acid (ALA) (Duke 1988). The applied concentrations of glyphosate to study the toxic effect on chlorophyll exerted a decrease in chlorophyll (a+b) content and this effect increased by increasing concentration (Fig. 3). The concentration 20 mM glyphosate inhibited the chlorophyll content and this inhibition ranged from 38 to 49% of the control culture during the incubation period. In this connection Saenz *et al.* (1997) indicated that chlorophyll a content of *Scenedesmus quadricauda* decreased significantly when it exposed to 50 mg Gly/L with respect to its control value. However, Wong (2000) indicated that the low concentration 0.02 mg/L stimulated the chlorophyll synthesis in *Scenedesmus quadricauda*, while higher concentrations were inhibitory.

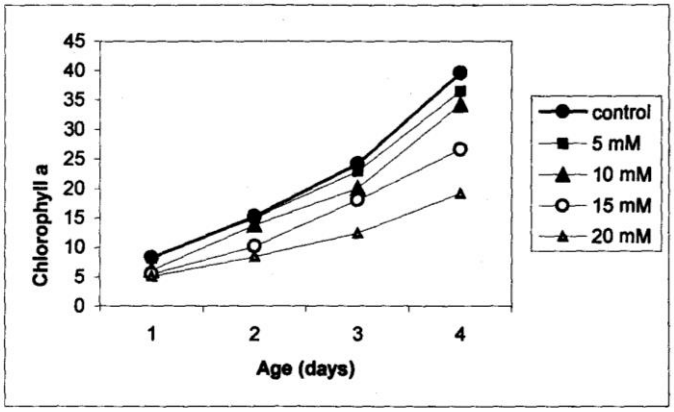

Fig. 3. Effect of glyphosate herbicide on chlorophyll content of *Chlorella kessleri* in mixotrophic culture. Each point is the mean of three different measurements.

Effect of Glyphosate on photosynthetic activity of *Chlorella*

In mixotrophic culture the photosynthetic activity of algae seems to be different than that grown in autotrophic culture in that the cells showed its maximum oxygen evolution after 48 hours (Table 1). The main reason is that in mixotrophic culture the cells of *C. kessleri* divide very quickly and the optical density increased and this prevent the light penetrate to the cells (self shading) and therefore oxygen evolution decreased. The photosynthetic activity of *C. kessleri* was affected by the application of glyphosate and the concentrations 15 and 20 mM inhibited the oxygen evolution. However, the same concentrations slightly increased the dark respiration. Goldsborough and Brown (1988) found that the application of less than 0.89 mg/L had no significant effect on the growth and photosynthesis of algae. The higher concentrations between 0.89 and 89 mg/L glyphosate significantly reduced the photosynthesis measured as carbon fixation. Van Rensen (1974) observed a 50% reduction in oxygen evolution by a culture of the green alga *Scenedesmus* sp. after 60-90 min exposure to $7x10^{-4}M$ (118 mg/L) glyphosate. He concluded from further studies of 2,6-dichloropenol indophenol (DCPIP) reduction in spinach chloroplasts that glyphosate directly inhibits photosynthetic electron transport in photosystem II. However, this was disputed (Richard *et al.* 1979) as a consequence of concomitant pH change in weakly buffered media. Glyphosate is known to be weakly acid and can release hydrogen ions (Sprankle *et al.* 1975).

Effect of glyphosate on protein content of *Chlorella*

From the results obtained in Table (2), it is obvious that glyphosate at low concentrations did not affect the protein content of *C. kessleri*. However, at the higher concentrations 20 mM, the inhibition was ranged between 20 and 30% of control during the incubation period. The low inhibitory effect may be due to defence mechanism of the organism to accumulate or secretes some enzymes to balance this toxic effect. Smart *et al.* (1985) indicated that when the plant cells are subjected to high glyphosate concentrations,

they increased extractable activity of the shikimate pathway enzyme 5-enol-puruvylshikimate-3-phosphate (EPSP) synthase.

Table 1. Effect of different concentrations of glyphosate herbicide on the photosynthetic activity of *Chlorella kessleri*. The photosynthetic activity measured as oxygen evolution or oxygen uptake (dark respiration) and the results are expressed as µmol O_2.mg $Chl^{-1}.h^{-1}$.

Age	Control		5 mM		10 mM		15 mM		20 mM	
(h)	+ O_2	- O_2	+ O_2	- O_2	+ O_2	- O_2	+ O_2	- O_2	+O_2	- O_2
24	80	91	75.6	86	61.9	81	46	101	41	116
48	130	112	114	94	103	103	55	111	67	134
72	95	115	89	105	100	102	89.9	114	75	150
96	64	120	56	117	99.7	104	128	128	88	166

Table 2. Protein content of Chlorella kessleri as affected by different applied concentrations of the herbicide glyphosate (mg/gm dry weight). ± SE of the mean of three replicates.

Time (hrs)	Control	5 mM	10 mM	15 mM	20 mM
24	159 ±3.6	151 ±3.3	153 ±4.2	130 ±1.1	126 ±3.3
48	372 ±51	331 ±25	331 ±45	245 ±19	273 ±15
72	405 ±29	395 ±14	355 ±41	330 ±22	295 ±22
96	601 ±37	582 ±19	555 ±7.6	517 ±12.8	416 ±28

Effect of glyphosate on amino acids content of *Chlorella*

Interference with the biosynthesis of aromatic amino acids has been postulated as the mode of action of the nonselective, broad spectrum herbicide glyphosate in higher plants as well as microorganisms (Jaworski, 1972). In the present work the amino acid content of the control and the algae treated with different concentrations of glyphosate was determined after the incubation period (96 hrs.). The glyphosate inhibited the biosynthesis of the shikimate pathway amino acids as well as kreb's cycle. However, all concentrations used increased the amino acid content of the triose-puruvic acid family as compared with the control cells (Table 3). At concentration 20 mM the amino acid of glutamate family inhibited by about 90%, aspartate family by 96% and the kreb's cycle family by about 94%. The total amino acids of the alga was inhibited by 30% at concentration 20 mM as compared with the control.

Effect of adding the aromatic amino acids on growth and amino acid content of *Chlorella*

From the results obtained above, it was found that concentration of 20 mM glyphosate was the most toxic one for the alga. In the previous work concerning the mode of action of glyphosate, a relationship with the biosynthesis or metabolism of aromatic amino acids was indicated. These phenomena were noticed in *Rhizobium japonicum* (Jaworski, 1972), *Escherichia coli* (Gresshoff, 1979) and *Chlamydomonas reinhardtii* (Jaworski, 1972), and Carrot cell culture (Killmer *et al.* 1981). Therefore in this work, the aromatic amino acids phenylalanine, tryptophane and tyrosine (10 mg/ml) were added to the *Chlorella* culture treated with 20 mM glyphosate and the growth and amino acids

content were estimated (Table 3 and Figure 4). The inhibition of growth by 20 mM glyphosate ranged between 51-60% of the control during the incubation period. When the three aromatic amino acids were supplemented in the culture medium, the inhibition was ranged between 17-26% only. The recovery effect was more higher at the end of the incubation period (72-96 hrs), than that at the beginning of incubation. The results obtained are in agreement to some extent with the results obtained with other plant cell cultures (Haderlie *et al.* 1977; Gresshoff, 1979), that the effect of glyphosate can be reversed by a combination of phenylalanine, tryptophane and tyrosine in the culture medium, indicating that inhibition of growth by glyphosate results from inhibition of aromatic amino acids synthesis. Smart *et al* (1985) obtained 0.0 inhibition of growth of *Corydallis sempervirens* by 5 mM glyphosate after addition of the three aromatic amino acids, however, in the present work the inhibition was 17% after the addition of the three aromatic amino acids but in the presence of higher concentration (20 mM) of glyphosate. In conclusion it is obvious that glyphosate herbicide affected the growth, photosynthesis and chlorophyll content of the green alga *Chlorella kessleri.* The inhibition is concentration dependent. Glyphosate also inhibits the amino acid content of the alga and severely affected the amino acids of glutamate and aspartate family of kreb's cycle. The recovery of the inhibition by addition of the aromatic amino acids conclude also that glyphosate affect these amino acids synthesis and it can be also concluded that glyphosate affect glutamate and tricarboxylic acid cycle. This conclusion is in accordance with Killmer *et al.* (1981) as they reversed glyphosate effect by addition of glutamate and intermediates of the tricarboxylic acid cycle.

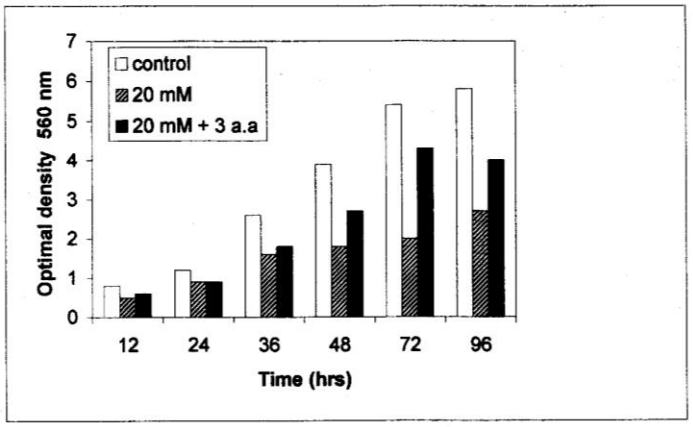

Fig. 4. Effect of addition of the aromatic amino acids phenyl alanine, tryptophane, and tyrosine (10 mg/ml) on growth of *Chlorella kessleri* in the absence and presence of 20 mM glyphosate herbicide.

Table 3. Effect of different concentrations of glyphosate herbicide on amino acids contents (mg/g dry weight) of *Chlorella kessleri* and the recovery effect after the addition of 10 mg/ml tryptophane, tyrosine and phenylalanine amino acids (3 aa).

	Kreb's cycle family												Triose-puruvic acid family									Shikimic acid family			
	Glutamate family					Aspartate family							Triose family				Puruvate family								
	Glu	Arg	Pro	His	Total	Asp	Thr	Lys	Isolu	Meth	Total	Total	Gly	Ser	Cyst	Total	Ala	Val	Leu	Total	Total	Ph.ala	Tyr	Total	Total
Control	2	11	5	1	19	7	24	8	7	5	51	70	-	-	3	3	7	1	14	22	25	6	11	17	112
5 mM	-	4	-	1	5	-	20	4	7	-	31	36	-	40	2	42	24	2	15	41	83	1	3	4	123
10 mM	-	68	-	50	118	2	-	2	-	-	4	122	1	3	32	36	3	-	5	8	44	1	8	19	175
15 mM	23	7	17	16	63	5	15	11	-	-	31	94	-	-	10	10	14	17	-	31	41	1	3	4	139
20 mM	-	1	1	-	2	-	-	-	-	2	2	4	-	9	15	24	3	29	2	34	58	3	5	8	70
20 mM+3aa	55	-	41	50	146	34	-	1	23	42	100	246	27	-	86	113	15	2	5	22	135	7	10	17	398

References

Alberdi, J.L., Saenz, M.E., Di Marzio, W.D. and **Tortorelli, M.C.** 1996. Comparative acute toxicity of two herbicides, paraquat and glyphosate, to *Daphnia magna* and *D. spinulata. Bull. Environ. Contam. Toxicol.* **57: 229-235.**

Atkinson, D. 1985. In: The herbicide glyphosate. (Grossbard, E. and Atkinson, D. eds.) Butterworths, London. pp **127-133.**

Barrett, P.R.F.1985. In: the herbicide glyphosate. (Grossbard, E. and Atkinson, D. eds.) Butterworths, London.

Becerril, J.M., Duke, S.O. and **Lydon, J.** 1989. Glyphosate effects on shikimate pathway products in leaves and flowers . *Phytochem.* **28: 695-699.**

Bowmer, K.H. 1982. Adsorption characteristics of seston in irrigation water: implications for the use of aquatic herbicides. *Aust. J. Mar. Freshw. Res.* **33: 443-458.**

Bradford, M, M. 1976. A rapid and sensitive method for the quantification of micrograms quantities of protein utilizing protein dye binding. *Anal. Biochem.* **72: 248-254.**

Cole, D.J., Dodge, A.D. and **Caselly, J.C.** 1980. Some biochemical effects of glyphosate on plant meristems. *J.Exper. Bot.* **31: 1665- 1674.**

Come, R.D., Bruns, V.F. and **Kelly, A.D.** 1976. Residues and persistence of glyphosate in irrigation water. *Weed Sci.* **24: 47-51.**

Duke, S.O. 1988. Glyphosate. In: Kearney, P.C. and Kaufman, D.D. (eds.) Herbicides: Chemistry, degradation and mode of action. Marcel Dekker, New York. pp. **1-70.**

Duke, S.O. and **Hoagland, R.E.** 1985. In: The herbicide glyphosate. (Grossbard, E. and Atkinson, D. (eds.) Butterworths, London.

Duke, S.O., Hoagland, R.E. and **Elmore, C.D.** 1980. Effects of glyphosate on metabolism of phenolic compounds . V. L-α-aminoxy-β-phenylpropionic acid and glyphosate effects of phenylalanine ammonia-lyase in soybean seedlings. *Plant Physiol.* **65: 17-21.**

El-Sheekh, M.M. 1999. Stable transformation of the intact cells of the unicellular green alga *Chlorella keslleri* with high velocity microprojectiles. *Biol. Plant.* **42: 209-216.**

El-Sheekh, M.M., Kotkat, H.M. and **Hammouda, O.H.E.** 1994. Effects of atrazine herbicide on growth, photosynthesis, protein synthesis, and fatty acid composition in the unicellular green alga *Chlorella kessleri. Ecotoxicol. Environ. Safety* **29: 349-358.**

Gardner, S.C.; Grue, C.E., Grassley, J.M., Lenz, L.A., Lindenauer, J.M. and **Seeley, M.E.** 1997. Single species algal (*Ankistrodesmus*) toxicity with Rodeo and Garlon 3A. *Bull. Environ. Contam. Toxicol.* **59: 492-499.**

Goldsborough, L.G. and **Brown, D.J.** 1988. Effect of glyphosate (Roundup formulation) on periphytic algal photosynthesis. *Bull. Environ. Contam.Toxicol.* **41: 253-260.**

Gresshoff, P.M. 1979. Growth inhibition by glyphosate and reversal of its action by phenylalanine and tyrosine. *Aust. J. Plant Physiol.* **6: 177-185.**

Harris, E.H. 1989. The *Chlamydomonas* source book.: A comperhensive Guide to Biology and Laboratory use (Academic Press, San Diego), pp. **780.**

Haderlie, L.C, Widholm, J.M., and **Slife, F.W.** 1977. Effect of glyphosate on carrot and tobacco cells. *Plant Physiol.* **60: 40-43.**

Hoagland, R.E., Duke, S.O. and **Elmor, C.D.** 1978. Effects of glyphsate on metabolism of phenolic compounds II Influence on soluble hydroxyphenolic compound, free amino acid and soluble protein levels in dark grown maize roots. *Plant Sci. Lett.* **13: 291-299.**

Jaworski, E.G. 1972. Mode of action of N-phosphonomethyl-glycine inhibition of aromatic amino acid biosynthesis. *J. Agric. Food Chem.* **20: 1195-1198.**

Killmer, J., Widholm, J. and **Slife, S.** 1981. Reversal of glyphosate inhibition of carrot cell culture growth by glycolytic intermediates and organic and amino acids. *Plant Physiol.* **68: 1299-1302.**

Laber, B., Kiltz, H.H. and **Amerhein, N.** 1986. Inhibition of phenylalanine ammonia-lyase *in vitro* and *in vivo* by (1-amino 2-phenylethyl) phosphonic analogue of phenylalanine. *Z.Naturforsch.* **41c: 49-55.**

Leganes, F., Sanchez-Maeso, E. and **Fernandez-Valiente, E.** 1987. Effect of indolacetic acid on growth and dinitrogen fixation by blue-green algae. *Sven. Bot. Tidskr.* **64: 460-461.**

Lichtenthaler, H.K. and **Wellburn, A.R.** 1983. Determination of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **603: 591-592.**

Moore, S., Spachman, D.H. and **Steins, W.** 1958. Chromatography of amino acids on sulphonated polysterene resins. *Anal. Chem.* **30: 1185-1190.**

Newton, M., Howard, K.M., kelpsas, B.R., Danhaus, R., Lottman, C.M. and **Dubelman, S.** 1994. Fate of glyphosate in an Oregon forest ecosystem. *J. Agric. Food. Chem.* **32: 1144-1151.**

Peterson, H.G., Boutin, C., martin, P.A., Freemark, K.E., Ruecker, N.J., and **Moody, M.** 1994. Aquatic phytotoxicity of 23 pesticides applied at expected environmental concentrations. *Aquatic Toxicol.* **28: 275-292.**

Richard, E.P. Jr., Gross. G.R. and **Arntzen, C.J.** 1979. Glyphosate does not inhibit photosynthetic electron transport and phosphorylation in pea (*Pisum sativum*) chloroplasts. *Weed. Sci.* **27: 684-688.**

Roberts, F., Roberts, C.W., Johnson, J.J., Kyle, D.E., Krell, T., Coggins, J.R. , Coombs, G.H., Milhous, W.K, Tzipori, S., Ferguson, D.J.P., Chakrabarti, D. ***and*** **McLeod, R.** 1998. Evidence for the shikimate pathway in apicomplexan particles. *Nature* **393: 801-805.**

Saenz, M.E., Di Marzio, W.D., Alberdi, J.L., and **del Carmen Tortorelli, M.** 1997. Effect of technical grade and a commercial formulation of glyphosate on algal population growth. *Bull. Environ. Contam. Toxicol.* **59: 638-644.**

Smart, C.C., Johaenning, D., Mueller, G. and **Amrhein, N.** 1985. Selective overproduction of 5-enolpyruvyl shikimic acid -3-phosphate synthase in a plant cell culture which tolerate high doses of herbicide glyphosate. *J.Biol. Chem.* **260: 16338-16346.**

Sprankle, P., Megitt, W.F., and **Penner, D.** 1975. Adsorption, mobility, and microbial degradation of glyphosate in the soil. *Weed Sci.* **23: 229-234.**

Van Rensen, J.S. 1974. Effects of N-(phosphonomethyl) glycine on photosynthetic reactions in *Scenedesmus* and in isolated spinach chloroplasts. In: Avron, M. (ed) Proceedings of the third international congress on photosynthesis. Elsevier Publishing Co., Amsterdam. P **683**.

Wetherell, D. F. 1961. Culture of fresh water algae in enriched natural sea water. *Physiol. Plant.* **14: 1-6.**

Wong, P.K. 2000. Effects of 2,4-D, glyphosate and paraquat on growth, photosynthesis and chlorophyll-a synthesis of *Scenedesmus quadricauda* Berb 614. *Chemosphere* **41: 177-182.**

تأثير المبيد العشبى جليفوسات على النمو ، البناء الضوئى وبعض الأنشطة الأيضية فى الطحلب الأخضر كلوريلا كيسلراى

مصطفى محمد الشيخ

قسم النبات – كلية العلوم – جامعة طنطا – مصر

فى هذا البحث تم دراسة التأثير السام لمبيد العشب جليفوسات على النمو ، البناء الضوئى ، الكلوروفيل ومحتوى الأحماض الأمينية فى الطحلب الأخضر كلوريلا كيسلراى . وقد أوضحت النتائج أن التركيزات المنخفضة وهى ٥ و ١٠ ملى مولار من هذا المبيد كانت غير سامة على الطحلب بينما كانت التركيزات العالية وهى ١٥ و ٢٠ ملى مولار كانت أكثر سمية . كما أوضحت النتائج أن التركيز ٢٠ ملى مولار قد عمل على تثبيط دالات النمو المختلفة بحوالى ٥٠ ٪ إذا ما قورنت بالتجربة الضابطة بينما لم تؤثر التركيزات المنخفضة عن هذا التركيز تأثير ساماً على النمو ، البناء الضوئى و المحتوى الكلوروفيلى . وعند اضافة الأحماض الأمينية الأروماتية وهى فينيل ألانين ، تريبتوفان وتيروزين إلى وسط النمو الذى يحتوى على ٢٠ ملى مولار جليفوسات أمكن للطحلب استعادة النمو بنسبة ٣٤٪ خلال فترة التجربة . وقد تم تقدير محتوى الأحماض الأمينية لكل من المزارع الطحلبية و تشتمل على التجربة الضابطة ومزرعة معاملة بـ ٢٠ ملى مولار جليفوسات وأخرى معاملة بـ ٢٠ ملى مولار جليفوسات اضافة إلى الثلاثة أحماض الأمينية الأروماتية . وقد أوضحت النتائج أيضاً أن إضافة الأحماض الأمينية الأروماتية إلى المزرعة الطحلبية المعاملة بواسطة ٢٠ ملى مولار جليفوسات امكانية استعادة الطحلب للتصنيع الحيوى للأحماض الأمينية الأروماتية وكذلك الأحماض الأمينية الأخرى . وعلى ضوء هذه النتائج تمت مناقشة ما إذا كان تأثير المبيد العشبى جليفوسات فى طحلب الكلوريلا عكسى أم غير عكسى بعد اضافة الأحماض الأمينية الأروماتية التى ثبت ايقاف تصنيعها عن طريق الجليفوسات.