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Growth and Some Physiological Aspects of *Nostoc muscorum* in Response to Mixtures of Two Triazine Herbicides

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Herbicides were accumulated in irrigation canals and drains as a result of their massive use in agriculture and weed control (Morris and Ron 1981). Triazine herbicides inhibit algal growth (Frank et al. 1982) and are toxic by disrupting photosynthesis (Stratton 1987). The range of inhibitory actions depend on the kind of herbicide, its dose, time of exposure and the individual characters of the organism (El-Dib et al. 1989).

Living organisms are more often exposed to multiple stresses in a complex biotype than to a single toxicant in a pure medium. It is important to determine the impact of mixed toxic substances in the environment (Bois et al. 1986). The present investigation was initiated to examine the effect of two triazine compounds namely, gardoprim and terbutryn singly and in combination on the growth criteria and nitrogen metabolism of the cyanobacterium Nostoc muscorum.

MATERIALS AND METHODS

The cyanobacterium Nostoc muscorum as a common alga in the Nile River water, was used as test organism in this study. Axenic cultures were grown in sterile Watanabae medium (El-Nawawy et al. 1958). Bioassay flasks were supplied with 500 ml of algal suspension and were incubated at 25°C ± 2 under continuous white fluorescent light (3000 Lux). The two triazines, gardoprim (2,3-diamine, N–(1,1–dimethyl-ethyl) N'–ethyl, 6-chloro-S-triazine and terbutryn (2,4-diamine-N'-(1,1-dimethyl-ethyl)-N'–ethyl-6(methyl thio)-S-triazine) (99.9% purity) were obtained from CIBA-Geigy (Swizerland). Solutions of each herbicide were prepared in methanol. The applied concentrations of the studied herbicides singly or in combinations were as follows: gardoprim (G): 50, 80, 200, 450 and 1010 µg/L; terbutryn (T): 18, 40, 88, 425 and 937 µg/L; mixture ratios : 1010 G + 18T(I); 200 G + 88 T(II); 50 G+ 937 T (III); 50 G + 18 T(IV) and 1010 G+ 937 T (V) µg/L. Three replicates from each treatment and control were used. Samples of algal suspensions (known volume) were withdrawn after each treatment at zero time, 1st, 3rd, 7th and 10th days. For each sample, growth was determined by dry weight (Skowronski et al. 1988). Chlorophyll (a) [chl (a)] was extracted

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using hot 100% methanol (Sartory and Grobbelaar, 1984). Chl (a) content was spectrophotometrically measured and calculated according to Amer. Publ. Health Assoc. (1985, p. 1069). At the 7th d when the maximum growth was attained, protein-N content was determined according to Qian and Wang (1989). Total nitrogen content was estimated according to Naguib (1969). Total carbohydrate content was measured as glucose (Dubois et al. 1956). For herbicide residue measurements a known volume (250 ml) was withdrawn from the aqueous media at the 7th d and extracted three times with 50 ml portions of 15% methylene chloride in hexane. Extracts were analyzed by GC fitted with an electron capture detector (Ni_{63}), and a stainless steel column packed with 1.5% OV – 17 + 1.95 OV – 210 coated on 80/100 cromosorb W. The column injector and detector temperatures were 190, 220 and 270°C, respectively. Nitrogen as a carrier gas, was used at a flow rate of 30 ml/min. Samples were run in triplicate with blank. Calibration program was verified by measurement of one or more concentrations of each standard. Ouality control sample was analysed with the actual samples. The relative standard deviation ranged between 10% to 15% of the mean.

The tested herbicide concentrations which reduced algal Chl(a) content by 50% (EC_{50}) were determined using probit method (Finney 1971) and growth rates were calculated according to Amer.Publ.Health Assoc. (1985).
Growth rate = (ln Chl (a) at T2- In Chl (a) at T1) / T2-T1) where T1 and T2 are the starting and ending time in days.

RESULTS AND DISCUSSION

The effects of gardoprim, terbutryn and their mixtures on the growth of Nostoc muscorum were recorded in Fig.1. Marked inhibitory effect on biomass yield was observed as the concentration of gardoprim or terbutryn increased. Gardoprim only stimulated algal growth at 50 µg/L, contributing 105% of control at the 7th d of incubation, whereas terbutryn at 18 µg/L level stimulated growth by 105 and 120% of control at the 1st and 3rh days of incubation. Lower, moderate and higher concentrations from a mixture of the two triazines resulted in gradual decrease in dry weight reached to 82%, 74% and 43% of control respectively. Such a trend coincides with the inhibitory effects of both triazines in their mixture on the growth rate (Table 1) and metabolic activities in Nostoc cells (Fig. 2). Reduction in algal dry weight in mixture I and III indicates that terbutryn is more inhibitory to Nostoc cells than gardoprim when supplemented in combination.

Gardoprim and terbutryn at all concentration levels decreased Chl (a) content of Nostoc cells (Fig.1). The inhibitory effect was initiated subsequently after 24 hrs of experiment reaching its highest stress at the end of incubation period. These observations are in agreement with those of (Cox 1983), who reported that atrazine beyond 0.1 mg/L caused decrease in Chl (a) content in phytoplankton. Also, El-Dib et al. (1989) concluded that gardoprim at 0.05 to 0.2 mg/L and

terbutryn at 0.005 to 0.05 mg/L decreased Chl (a) content of Scenedesmus sp. Accordingly, one may reach a conclusion that cyanobacteria are more resistant to both triazines than green algae. On the contrary. Stratton (1984) and Herman et al. (1986) suggested that blue-greens are more susceptible to atrazine than other algal groups. Following application of mixtures I, II and IV, a clear antagonistic effect was noticed. Mixtures III and V, however induced an additive effect on algal growth (Table 2). Schultz and Allison (1979) suggested that it is not possible to add toxic effects of two toxicants (pyridine and aniline) on Tetrakymenapyriformis as measured individually.

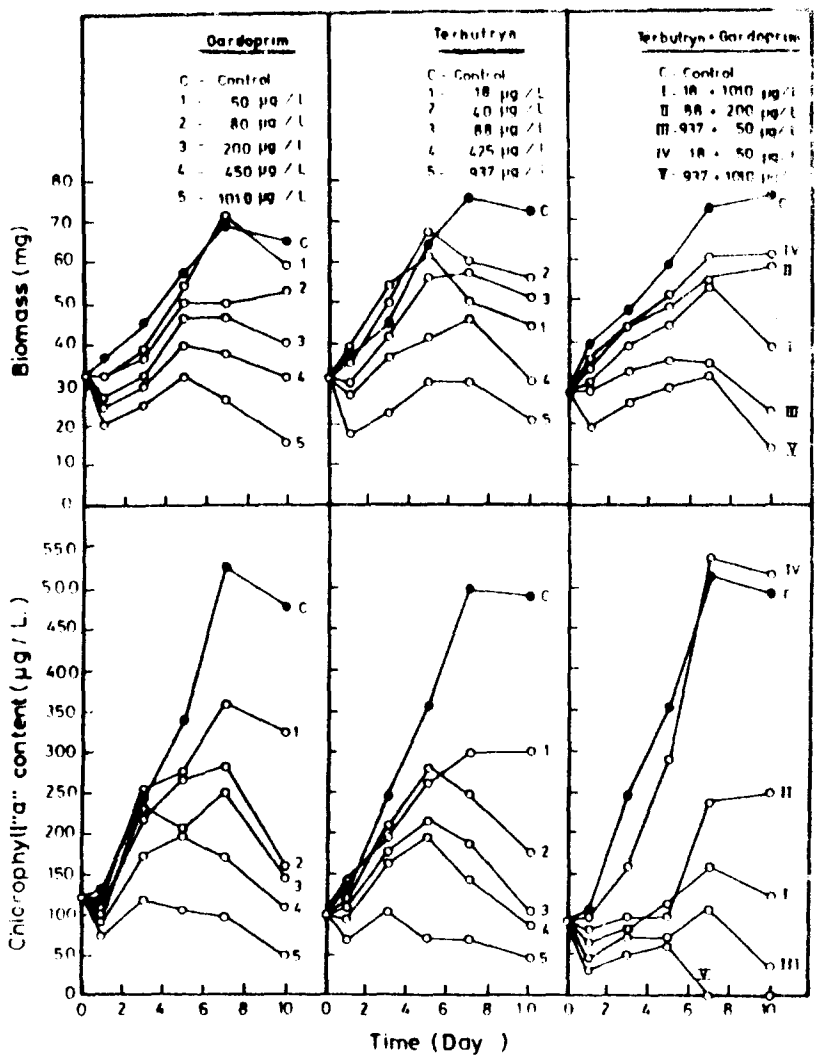

Figure 1. Effect of triazines on growth of Nostoc muscorum (Biomass and Chl(a)).

Available results revealed that gardoprim and terbutryn at higher concentrations supressed the growth rate of Nostoc cells (Table 1). Maximum growth rates were recorded at the 1st d of incubation and decreased gradually to reach its lowest value at the 10th d. Interaction of herbicide mixtures I, II, III and IV revealed clear antagonistic effect while at mixture V the growth rate was hardly detected indicating an additive effect. A consistent relationship could exist between herbicide concentration and the growth rate with respect to time. Such phenomenon was detectable after 3 days with gardoprim (r2–0.95); and after 5 days with terbutryn

Table 1. Growth rates of N. muscorum treated with gardoprim, terbutryn and mixture of them measured as chlorophyll (a) content.

Concentrations µg/L	Growth rate over time (days)				
	0–1	0–3	0–5	0–7	0–10
			Gardoprim		
Control	0.03	0.22	0.20	0.21	0.14
50	- 0.20	0.24	0.16	0.16	0.10
80	- 0.05	0.19	0.16	0.12	0.10
200	- 0.17	0.21	0.10	0.10	0.01
450	- 0.31	0.11	0.09	0.05	- 0.01
1010	- 0.49	- 0.01	- 0.03	- 0.06	- 0.08
r^{2**}	0.94	0.95	0.94	0.92	0.79
Intercept	- 0.02	0.23	0.17	0.16	0.08
Slope	- 0.05	- 0.24	- 0.20	- 0.23	- 0.18
			Terbutryn		
Control	0.25	0.30	0.25	0.236	0.16
18	0.32	0.22	0.19	0.15	0.11
40	0.19	0.24	0.20	0.13	0.06
88	0.09	0.19	0.15	0.09	0.003
425	- 0.04	0.16	0.14	0.03	- 0.02
937	0.36	0.02	- 0.08	- 0.06	- 0.08
r^{2**}	0.65	0.90	0.92	0.83	0.70
Intercept	0.21	0.25	0.22	0.16	0.09
Slope	- 0.03	- 0.25	- 0.30	- 0.24	- 0.20
			Gardoprim + Terbutryn		
Control	0.10	0.32	0.27	0.25	0.17
I	- 0.38	- 0.06	0.04	0.08	0.03
II	- 0.15	0.002	0.01	0.14	0.10
III	- 0.76	- 0.07	- 0.05	0.02	- 0.11
IV	0.05	0.18	0.23	0.25	0.17
V	- 0.12	- 0.21	- 0.09		
r^{2**}	0.09	0.56	0.66	0.81	0.53
Intercept	- 0.26	0.14	0.18	0.22	0.14
Slope	- 0.30	- 0.19	- 0.16	- 0.13	- 0.11

* Slopes are mean of three replicates.

** r^2 = Correlation coefficient of growth rate VS. treatment rate.

(r^2 = 0.92); however, the correlation decreased using the herbicide mixtures as indicated by conspicuous drop in r^2 (0.81) at the 7th d. Also the slopes for the relation between growth rate and herbicide concentrations declined over time. Reduction in the growth rate of cyanobacteria and algae by low concentrations of atrazine was reported by Stratton (1984) and Mayasich et al. (1986).

The EC_{50} value was calculated for each herbicide at 3,5,7 and 10 d after treatment. N. muscorum was more susceptible to terbutryn than gardoprim. The EC_{50} values generally decreased with time (Table 3).

Table 2. Residual concentration and percentage inhibition of Chl(a) in N. muscorum following gardoprim and terbutryn application.

Gardoprim Concentration µg/L			Terbutryn Concentration µg/L			Gardoprim + Terbutryn Concentration µg/L			
initial	residual	% inhibition*	initial	residual	%inhibition*	initial	residual G	residual T	% inhibition*
50	N.D.	30	18	N.D	40	I	730	N.D	69
80	16	46	40	9	51	II	6	N.D	53
200	58	52	88	23	62	III	N.D	260	79
450	200	68	425	150	76	IV	N.D	N.D	—
1010	590	84	937	460	86	V	920	630	100

* Chl(a) values compared with control.
N.D = Not detected.

Table 3. Toxic effect of herbicides on N. muscorum expressed as EC_{50} over time (mean of 3 replicates).

Herbicide	EC_{50} µg/L			
	3d	5d	7d	10d
Gardoprim	1000	630	130	60
Terbutryn	631	398	35	24

Lower and moderate concentrations of gardoprim and turbutryn induced significant increase in total carbohydrate accumulation in N. muscorum (Fig.2). Higher concentrations of both triazines individually caused significant inhibition in carbohydrate content with respect to their controls. All mixture concentrations used decreased total carbohydrate accumulation in Nostoc cells. The drop in total carbohydrate synthesis which matched with supression in Chl(a) synthesis at higher concentrations of herbicides may be attributed to a respective inhibition of photosynthesis. These observations are in harmony with the findings of Jones and Winchell (1984). According to the hypothesis by Devlin et al. (1983), an atrazine

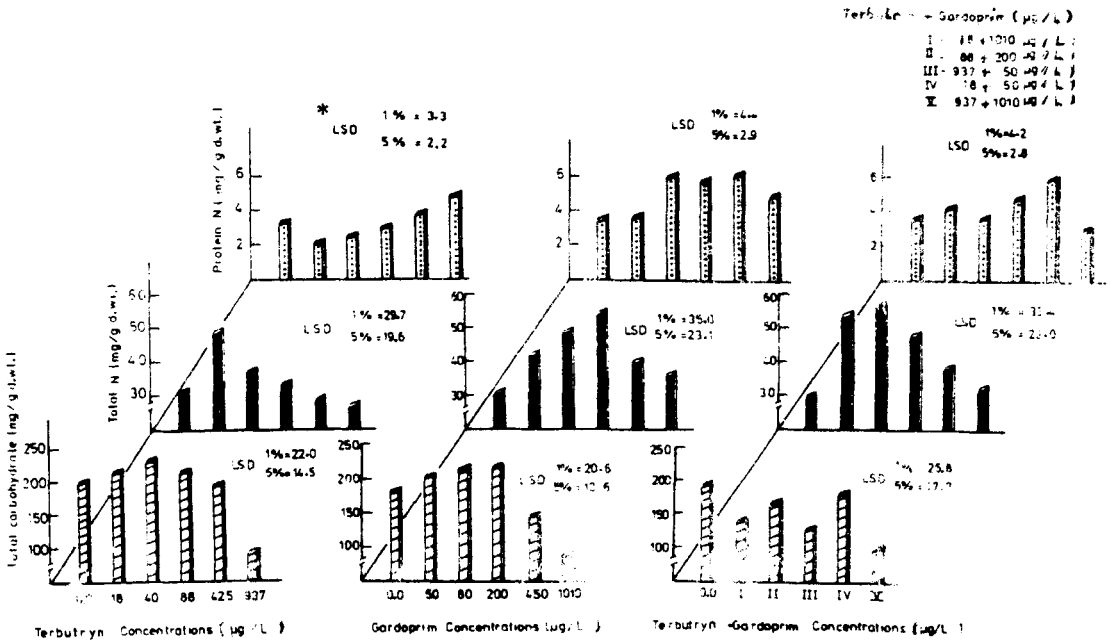

Figure 2. Effect of triazines on some metabolic aspects of Nostoc muscorum after ten days growth.

* LSD = Least significant difference between treatment and control at 1% and 5% levels.

blocked photosynthetic electron transport might explain the significant reduction in total carbohydrate accumulation with any further increase in herbicide concentration.

Protein nitrogen content increased as gardoprim concentration increased relevant to controls (Fig.2). On the other hand, terbutryn attenuated protein synthesis in Nostoc cells at lower and moderate levels. However, a slight elevation above that of control was noticed at 937 µg/L level. All mixture concentrations increased the protein content of algal cells except mixture V. The present data are in agreement with Shabana (1987), who reported that although atrazine is a metabolic inhibitor, yet it enhanced particularly the nitrogen metabolism leading to more amino and protein-N accumulation in Nostoc muscorum.At lower and moderate concentrations of both triazines, total nitrogen content recorded significant increase which indicates better nitrogen fixation process and metabolism of nitrogen. All herbicide mixtures used increased total nitrogen content in Nostoc cells. One may conclude from these data that the studied compounds affected the physiological and biochemical events which favor more utilization of carbohydrates for nitrate reduction and synthesis of amino acids and proteins.

Tracing the uptake of gardoprim, terbutryn and their mixtures by Nostoc cells indicated that maximum uptake levels were associated with relatively high growth rates, particularly at low herbicide concentrations (Tables 1,2). This may be attributed to the fact that increase in algal biomass is linked with the increase in surface area of algal cells and hence the rate of uptake. Such correlation was supported by the previous reports of Shehata et al. (1984) and El-Dib et al. (1989). The uptake decreased with increase in concentrations of the mixture showing a trend similar to that of the two triazines when added singly. Percentage pesticide uptake by cyanobacterial cells seemed to depend on the nature of pesticide and its concentrations in the culture.

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REFERENCES

American Public Health Association (1985) Standard methods for the examination of water and wastewater, 16th ed. Amer. Publ.Hlth.Assoc.,Washington, DC.

Bois F, Vaillant M, Vasseur P (1986) Multiple regression analysis of toxic interactions: Application to the microtox test and general comments. Bull Environ Contam Toxicol **36:** 707–714.

Cox H W (1983) The effect of 2,4–D, atrazine and diuron on algal physiology and population dynamics. Dissertation Abstracts International, B **43:** 3903–3904

Devlin R M , Murkowski A J , Zbiec I I , Karczmarczyk S J, Skorska E M (1983) Influence of buthidazole, diuron and atrazine on some light reactions of photosynthesis. Weed Sci., **31,** 6: 879–883.

Dubois M, Gilles K A, Hamilton J K, Repers P A, Smith F (1956) Colorimetric method for determination of sugars and related substances. Analyt Chem **18:** 350–356

E-Dib M A, Shehata S A, Abou Waly H (1989) Response of Freshwater alga Scenedesmus to triazine herbicides. Water, Air and Soil Pollution. **48:** 307–316.

El-Nawawy A S, Lotfi M, Fahmy M (1958) Studies on the ability of some bluegreen algae to fix atmospheric nitrogen and their effect on growth and yield of paddy. Agric Res Rev **36:** 308–320

Finney D, (1971) Probit analysis Cambridge. Cambridg e Univ Press, 1–333

Frank, D E N, Kettle W D, Donald E S (1982) The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States: Ecology, **63:** 1285–1293

Herman D, Kaushik N K, Solomon K R (1986) Impact of atrazine on periphyton in freshwater enclosures and some ecological consequences. Can J Fish Aquat Sci **43:** 1917–1925

Jones T W, Winchell L (1984) Uptake and photosynthetic inhibition by atrazine and its degradation products on 4 species-of submerged vascular plants. J Environ Qual **13:** 243–247

Mayasich J M, Karlander E P, Terlizzi D E (1986) Growth responses of Nannochloris oculata Droop and Phaeodactylum tricornutum Bohilin to the herbicide atrazine as influenced by light intensity and temperature. Aquat Toxicol **8:** 175–184

Morris K, Ron J (1981) Water Quality div, Oklahoma water resources broad Oklahoma city, Oklahoma 7315-Z J Aquat Plant Manage **19:** 15–18

Naguib M I (1969) On the colorimetry of nitrogen components of plant tissues. Bull Fac Sci Cairo Univ **43:** 1–5

Oian K, Wang S (1989) An improvement in determination of algal protein by the Folin phenol method. Haiyang YU Huzhao **20**(2): 192–196

Sartory D P, Grobbelaar J U (1984) Extraction of chlorophyll a from fresh water phytoplankton for spectrophotometric analysis. Hydrobiologia **114:** 177–187

Schultz T W , Allison T C (1979) Toxicity and toxic interaction of aniline and pyridine. Bull Environ Contam Toxicol **23:** 814–819

Shabana E F (1987) Use of batch assays to assess the toxicity of atrazine to some selected cyanobacteria. II-Effect of atrazine on heterocyst frequency, nitrogen and phosphorus metabolism of four heterocystous cyanobacteria. J Basic microbiol **27,** 4: 215–223

Shehata S A , Aly O A, Abou Waly H (1984) Effect of bladex, etazine, dicryl and baylucide on the growth of Scenedesmus. Environ Intl. **10:** 225–234

Skowronski T, Pawlick Barbara, Jakubowski M (1988) Reduction of cadmium toxicity to green micro alga Stichococcus bacllaris by manganese. Bull Environ Contam Toxicol **41:** 915–920

Stratton G W (1984) Effects of the herbicide atrazine and its degradation products, alone and in combination, on phototrophic microorganisms. Arch Environ Contam Toxicol **13:** 35–42

Stratton G W (1987) The effects of pesticides and heavy metals towards phototrophic organisms, In:Hodgson E (ed) Rev.In Environ Toxicol **3:**71–147