Egyptian J. of Phycol. Vol. 4(2), 2003

TOXICITY OF SUMI-ALPHA AND DURSBAN INSECTICIDES ON THE GROWTH VIABILITY, METABOLISM AND NITROGEN-FIXING CAPACITY OF TWO *NOSTOC* SPECIES.

Neveen Abdel-Raouf
Botany Dept., Fac. of Sci., Cairo Univ. (Beni-Suef Branch), Beni-Suef, Egypt.

Abstract

Two *Nostoc* species were used to study the toxic effects of two commonly used insecticides: sumi-alpha [(s)-α-cyano-3-phenoxybenzyl (s)-2- (4-chlorophenyl)-3-methylbutyrate] and Dursban [O,O-diethyl-O-(3,5,6-trichloro-2-Pyridyl) phosphorothioate)] to soil microflora. The heterocystic filamentous *Nostoc minutum* Desm. ex. Born. *et* Flah. & *Nostoc puniforme* Ag. ex. Born. *et* Flah. were exposed to different concentrations of sumi-alpha and dursban. Differential growth effects were observed on the two cyanobacterial species exposed to different insecticide concentrations. Selected parameters of the tested cyanobacteria (dry weight, Chl *a*, photosynthetic O_2 evolution, total soluble sugars, nitrogenase activity, total soluble proteins, total lipids and fatty acid contents of the total lipids) were measured and examined. The data revealed a marked inhibitory effects in all measured parameters in both *Nostoc* species under different insecticides concentrations of sumi-alpha and dursban except the total lipids. This suggests a potential hazard of these insecticides on soil microflora and subsequently natural ecosystem.

Key words, Cyanobacteria , Insecticide Toxicity, Sumi-alpha, Dursban.

Introduction

Much more attention has been paid to the effects of various insecticides on microorganisms. The contamination of aquatic environments with both sumi-alpha and dursban insecticides is a long-term problem because of the chemical stability and potent toxicity of these pollutants. Of the many organophosphorous (dursban) and pyrethrins (sumi-alpha) are among the most ubiquitous and are the most prominent contaminants in the aquatic biota. Contamination of aquatic habitats by sumi-alpha and dursban is an unavoidable characteristic of aerial spraying of forests. These insecticides enters the aquatic system primarily via direct application by spray air craft or indirectly from spray drift and surface runoff (Abd-El Hafez, *et. al.*, 1996).

In Egypt the surface-water concentrations of studied insecticides ranged from 1L to 600mL per Fadan for both dursban and sumi-alpha, respectively. Several detailed reviews (Kunert & Boger, 1981, Nikolenko, and Amirkhanov, 1993, and Kent & Caux, 1995) addressed sumi-alpha and dursban degradation and persistence in aquatic environments. These provide the necessary context for their exposure, duration profile and subsequent potential toxic hazard to phytobiota.

(ISSN: 1110-8649)

Field studies have demonstrated bioaccumulation and persistence of these insecticides in sediments and particularly in aquatic plants (Ashton & Crafts, 1981, Abd-ElHafez-Alia, *et al.*, 1996 and Ibraheem, 2002).

Due to a variety of factors (e.g. small size, high surface to volume ratios, limited cell barriers, high lipid content, and effective adsorptive capacity), it is expected that algae in general should possess a high capacity for sequestering and concentrating lipophilic pesticides from the ambient medium. The toxicity of organophosphorus and pyrethrins was studied also by Nikolenko & Amirkhanov, (1993). Blue-green algae, specially the nitrogen fixers cyanobacteria represent the major microorganisms which contribute soil fertility. These organisms play an important role in this system by providing a steady input of fixed nitrogen (Roger, *et. al.*, 1986).

Insecticides drastically influence all aspects of algal metabolism. Understanding the physiology and biochemistry of various insecticidal action will enable to make right management decisions at high and low using. In Egypt, dursban and sumi-alpha are the major applied insecticides to get rid of bollworms and other cotton pests. Higher concentrations of these insecticides were applied in different types of soil and water bodies. These insecticides had adverse effects on long term soil fertility, soil productivity, environmental quality and aquatic environment.

The aim of the present work was to ascertain phytotoxic effects of two insecticides belonging to different groups having varied modes of action on dry weight, chlorophyll *a* , carotenoids, the photosynthetic oxygen evolution, total soluble sugars, proteins, nitrogenase activity, lipids and fatty acids of the two investigated cyanobacteria. The insecticides tested were selected on the basis of recommendations of Ministry of Agriculture and land Reclamation, for the integrated control of cotton bollworms.

Materials and Methods

Cyanobacterial isolates

Two cyanobacterial species were isolated from cotton fields .*Nostoc minutum* Desm. ex. Born. *et* Flah. & *Nostoc puniforme* Ag. ex. Born. *et* Flah. Purification of algal isolates was carried out primarily by repeated culturing and subculturing on Allen's medium (1968 modification of Hughe's Gorthan and Zehnder's, 1958) until they were obtained final on pure unialgal cultures, which were then identified according Prescott (1951,1969 & 1978). The axenic isolates of algae were inoculated into 100 ml sterilized liquid medium having the same components as solid media contained in 250 ml conical flasks. These cultures were then left to grow under 27±2°C and light intensity 3000 Lux through light-dark cycle of 16-8 hrs respectively during the growth time, with optimum growth period (8 days).

Chemicals tested

Two insecticides, sumi-alpha {[O,O-diethyl-O-(3,5,6-trichloro-2-Pyridyl) phosphorothioate]} and dursban { (s)-α-cyano-3-phenoxybenzyl (s)-2- (4-chlorophenyl)-3-methylbutyrate} (kindly supplied from Agricultural Research center, Ministry of Agriculture, Egypt) were used in this investigation. Both insecticides were prepared in stock solutions and added aseptically to the culture media to the final concentrations indicated for each treatment. The applied insecticides concentrations were 0.02, 0.04, 0.06 and 0.08 % for sumi-alpha and 0.002, 0.004 , 0.008 and 0.012 % for dursban. The common and trade names, and the prescribed field application rates are given in Table 1.

Table (1). Detailed characters of the insecticides used.

Common name	Trade name	Chemical name	Dosage*/ Fadan
Chlorpyrifos	Dursban	[O,O-diethyl-O-(3,5,6-trichloro-2-Pyridyl) phosphorothioate].	1 Liter
S-fenvalerate	Sumi-alpha	(s)-α-cyano-3-phenoxybenzyl (s)-2-(4-chlorophenyl)-3-methylbutyrate.	600 ml

***Recommended dosage in 600 liters of water [Abd-El Hafez , *et al.,* (1996)].**

Measurement of algal growth

Dry weight determination

The culture masses of 8 days age (logarithmic growth phase) were separated by suction filtration, then dried at 90 ˚C till a constant weight was attained.

Determination of pigments. Chlorophyll *a* and carotenoids were estimated by the method recommended by Metzener, *et. al.*, (1965).

Oxygen evolution determination. Photosynthetic oxygen evolution was polarigraphically determined by following the changes in O_2 concentrations within three hours in the medium with a calibrated Clark type oxygen electrode (Ono and Murata 1981). Three ml aliquots of cell suspensions, with a mass density of 0.1mg ml^{-1},were placed in temperature controlled cuvette and illuminated with a quantum flux density of 300μ $Em^{-2}s^{1.}$

The Metabolic analyses.

All the following parameters were estimated at sharp logarithmic phase (8 day old culture)

1. Total soluble sugars. Total soluble sugars were determined using the modified methods of Yemm and Willis (1954) and Handel (1968), respectively.

2. Nitrogenase activity (acetylene reduction method). The cultures were grown without nitrogen source to estimate the nitrogen fixation ability of the two

studied cyanobacteria using acetylene reduction technique of Stewart *et al.*,(1971).

3. Total soluble proteins. It was carried out according to the method of Lowery *et al.,* (1951), using bovine serum albumin as a standard.

4. Total lipid content. quantitative determination of total cellular lipids using dichromate reduction method (Kochert's, 1978) was used.

5. Fatty acid content of total lipids. Replicate samples were harvested from control and treated cultures separated from the growth medium by centrifugation. Prior to this step, subsamples were taken for cell count and mass measurements. Using a vortex mixer, cells were rinsed three times with 10 mL of fresh growth medium to remove any loose surface material (i.e., gelatinous exogenous material). Extraction and purification of total lipids followed modifications of the established procedures (Caux, 1989; Bligh and Dyer, 1959 and Kates, 1972). After the rinse, total lipids were extracted by adding 8.0 mL methanol-chloroform-0.2 M hydrochloric acid solution (2:1:0.8, v/v/v), sonicating for 10 min, and leaving the suspension undisturbed at 22˚C for 1 h. The suspension was then centrifuged (700 r.p.m for 10 min).

The extraction procedure was repeated once on the debris pellet and the supernatants combined. Water and nonlipid contaminants were removed through a two-phase purification step. Chloroform (2.0 mL) and bidistilled water (2.0mL) were added to the combined supernarants to obtain a methanol chloroform water ratio of 1:1:0.9 (v/v/v). The mixture was agitated with a vortex and centrifuged (700 r.p.m for 10 min).The chloroform phase (bottom layer) was carefully removed with a Pasteur pipette and neutralized to pH 7.0 with 0.3 M NH_4OH in methanol. The purified lipids were concentrated under N_2, resuspended in chloroform (2.0 mL) and transferred to teflon-capped glass vials (4.0 mL) and stored under N_2 in the freezer. Total lipids were then transesterified and methylated (Metcalfe *et al.,* 1966) and the fatty acid methyl esters (FAME) were quantified by gas chromatography following an internal standard procedure (Grenier, *et al.,*1979) using methylheptadecanoate. A Hewlett Packard model 5880 gas chromatograph equipped with a hydrogen flame ionization detector was employed. FAME extracts were injected directly into a 2 mm i.d. glass column packed with Supelco GP SP-2330 on 100/120 Chromosorb WAW. Oven temperature was maintained at 175˚C. The injection port and detector temperatures were 210 and 240 ˚C, respectively. Peak areas were determined using a Hewlett Packard digital integrato model 5880A.

Statistical analyses. Analyses of variance (one-way ANOVA) was employed to determine if treatments were significantly different from each other (Zar, 1984). Results were seemed significantly different at the levels 5% & 1%. All experiments were repeated of four replicates.

Results and discussion

The assessment of the toxic effect of sumi-alpha and dursban on dry weight of both *Nostoc minutum* and *Nostoc puniforme* (Figs. 1 & 2) indicated a decrease in dry weight compared to control in the cultures exposed to all applied concentrations. In addition, *Nostoc minutum*. exhibited a gradual reduction in dry weight with the treatment by both insecticides (Fig. 1). At 0.08 and 0.012 % of sumi-alpha and dursban dry weights were inhibited (83.5 and 97.5 %) during 16 days, respectively (Fig. 1). Also, the dry weight of the Cyanobacterium *N. puniforme w*as affected by the application of the different concentrations of both applied insecticides (Fig. 2).

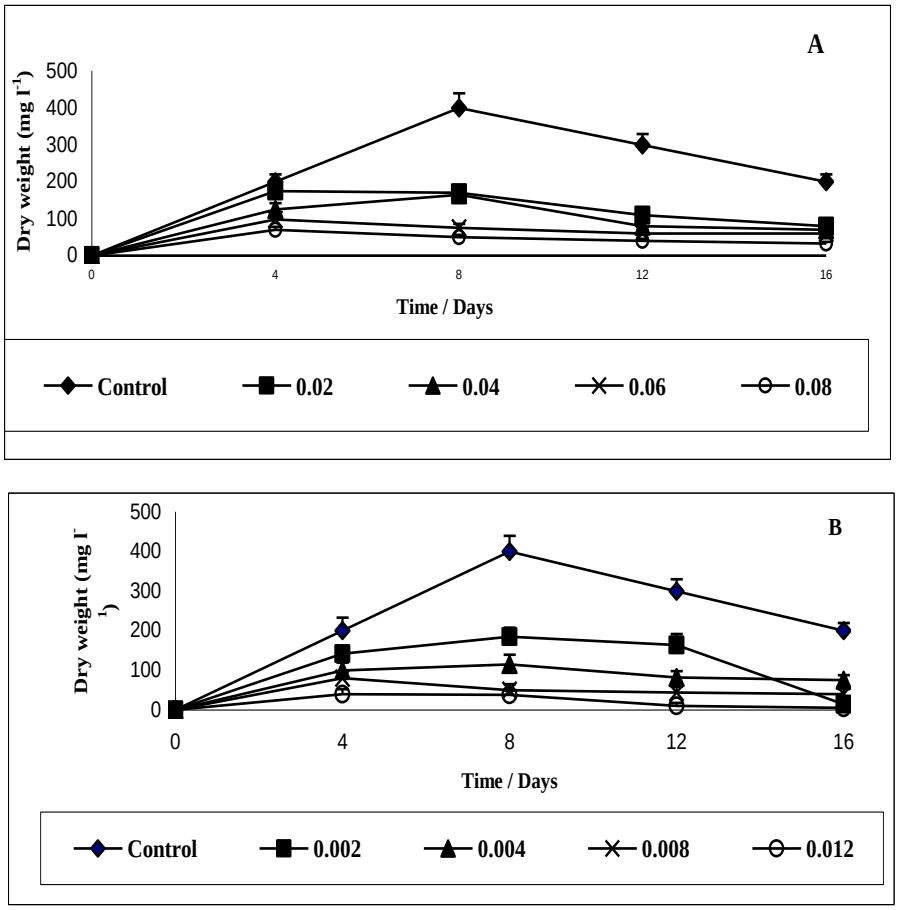
It is evident that by increasing the concentration to 0.08 % of sumi-alpha and 0.012 % of dursban, there was a decrease in growth of *N. puniforme*. The inhibition was maximum and accounted for about 95 and 98 % of control at the high concentrations (0.08 & 0.012 %) of sumi-alpha and dursban after 16 days, respectively. Several investigators also reported that algal growth was greatly affected by the application of several insecticides (Nikolenko & Amirkhanov, 1993 and Ibraheem, 2002). Growth reduction by insecticides could be generally caused by the inhibition of cell division. However, the reduction in dry weight of the two studied cyanobacteria could be attributed to physiological and biochemical disturbances resulted from the insecticide-induced stress (Kent & Caux, 1995, Khalifa-Neveen, 1999, and Ibraheem, 2002).

The present results showed that the two used insecticides generally reduced the contents of Chl *a* (Figs 3 & 4). The highest inhibitory effect was observed with high concentrations of dursban followed by sumi-alpha. This is in agreement with the results of several investigators (Batterton, *et al.,* 1971, Singh, 1974, Awad, 1978, and Nikolenko & Amirkhanov, 1993) who found that the application of low and high doses of dursban and sumi-alpha results in a decrease in the content of chl *a* in many algae.

Kunert and Boger (1981) indicated that herbicide-induced pigment bleaching might be caused either by inhibition of biosynthesis, leaving the present pigment level unchanged, and / or by pigment destruction. The phytotoxicity of some herbicides could be explained by their ability to cause abnormal accumulation of tetrapyrroles addition to the blockage of the prophyrin pathway, the precursors of chlorophyll biosynthesis. Consequently, this would inhibit the chlorophyll synthesis (Duke, *et al.,* 1991, Ricotta and Masiunas, 1992).

The amount of carotenoids in both cyanobacteria cells after treatment with insecticides are given in Table (2). It is obvious that, carotenoids content in both organisms decreased after treatment with insecticides as compared to control. The minimum values (0.1 and 0.2 mg L^{-1}) were recorded after treatment with (0.08 %) sumi-alpha at the end of experiment after (16 days) as compared to that of the untreated cultures (0.8 and 1.1 mg L^{-1}) for both *Nostoc*

minutum and *N. puniforme* , respectively. Also, treatment with dursban resulted in a gradual reduction in carotenoids content of both studied algae with residual concentrations. The highest decrease (87.5 and 89 %) was recorded at high concentration of dursban (0.012 %) for both *Nostoc minutum* and *N. puniforme*, respectively as compared with control.



Figure(1). Dry weight of *Nostoc minutum* treated with different concentrations of sumi-alpha (0.02-0.08 %) (A), and dursban (0.002-0.012 %) (B).

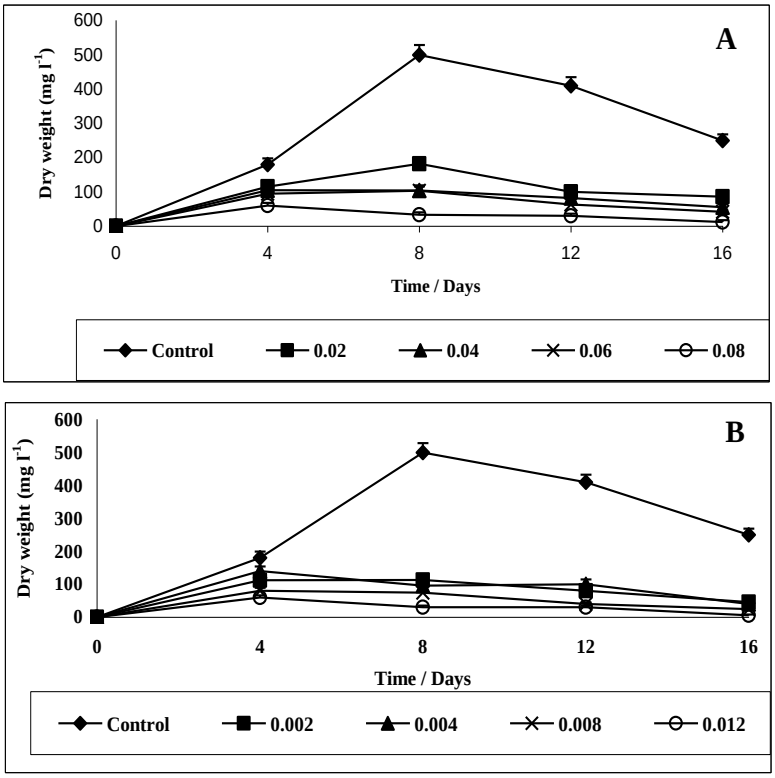


Figure (2). Dry weight of *Nostoc puniforme* treated with different concentrations of sumi-alpha (0.02-0.08 %) (A) and dursban (0.002-0.012 %) (B).

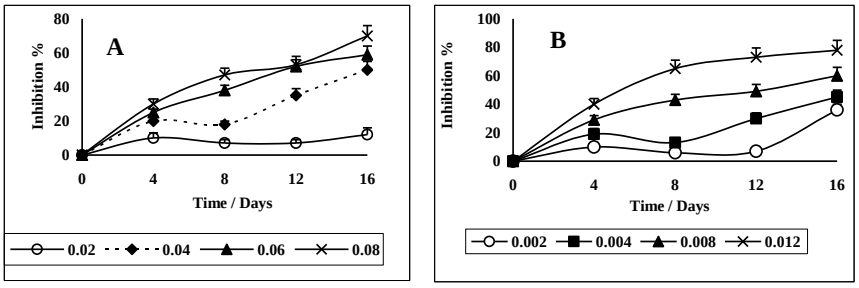


Figure (3). Inhibition percentage of chl *a* contents of *Nostoc minutum* treated by different concentrations of sumi-alpha (0.02-0.08 %) (A) and dursban (0.002-0.012 %) (B).

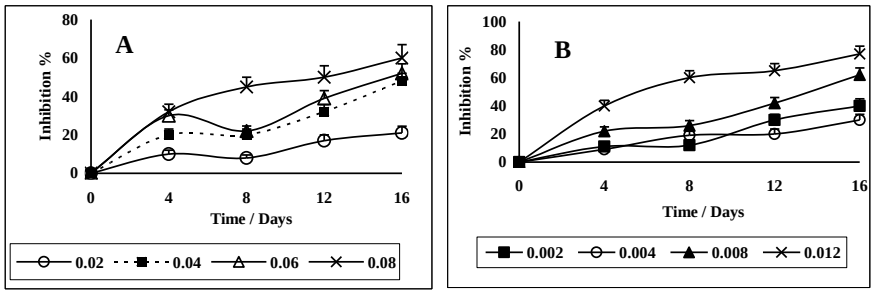


Figure (4). Inhibition percentage of chl *a* contents of *Nostoc puniforme* treated by different concentrations of sumi-alpha (0.02-0.08 %) (A) and dursban (0.002-0.012 %) (B).

The results of this investigation are in agreement with the results of several investigators (Ashton & Crafts, 1981 and Khalifa-Neveen, 1999) who found that the application of some insecticides resulted in a decrease in the content of carotenoids and chlorophylls in many algal species such as *Anacystis nidulans and Scenedesmus bijugatus*. Carotenoids were reported to be highly susceptible to insecticides attack where they are lost more rapidly than chlorophylls. It is of interest to note here that carotenoid pigments act as chemical buffer to protect the chlorophyll (Krinsky, 1967). Thus, a loss of chlorophyll protection by the carotenoid reduction could be a factor inducing diminution in chlorophyll content (Bartels & Waston, 1978).

The destruction effect of insecticides on both chlorophyll and carotenoid may be induced through the action of these insecticides on photosynthetic activity (Ashton & Crafts, 1981, Khalifa-Neveen, 1999, and Ibraheem, 2002).

The photosynthetic activity measured as O_2 evolution was inhibited by application of 0.06 and 0.08 % of sumi-alpha. The concentrations of 0.06 and 0.08 % inhibited O_2 evolution of *N. minutum* and *N. puniforme* by 60, 77, 63 & 95 % as compared with control. Also, the inhibition of O_2 evolution was maximum and accounted for about 80 % and 87 % of controls at the concentrations 0.012 % dursban on *N. minutum* and *N. puniforme* ,respectively.

The results in Fig. 5 indicated that sumi-alpha and dursban have relatively strong effect on O_2 evolution. It was found that results of O_2 evolution in accordance with the results of dry weight and chl *a* (i.e. the inhibition in all studied parameters were by increasing the concentration of insecticides). In this connection, Ibraheem (2002) reported that the dry weight, chl *a* and photosynthetic activity in two species of *Anabaena* showed marked inhibition with the treatment by larvin and sevin insecticides.

Table (2). Effect of Sumi-alpha and Dursban on carotenoid contents (ug ml^{-1}) of *Nostoc minutum* and *Nostoc puniforme* [Data were recorded at sharp logarithmic phase(8 days) and the mean ± SD of three replicates].

Insecticide concentration (%)		Carotenoids (µg ml^{-1}).	
		Nostoc minutum	*Nostoc puniforme*
0.0		0.80 ± 0.03	1.1 ± 0.1
Sumi-alpha	0.02	0.83 ± 0.02	0.6 ± 0.1
	0.04	0.70 ± 0.01	0.45 ± 0.2
	0.06	0.30 ± 0.01	0.24 ± 0.03
	0.08	0.20 ± 0.01	0.10 ± 0.01
Dursban	0.002	0.75 ± 0.1	0.53 ± 0.2
	0.004	0.53 ± 0.02	0.41± 0.03
	0.008	0.30 ± 0.1	0.19 ± 0.01
	0.012	0.11 ± 0.03	0.12 ± 0.01
LSD between rows at 5%		0.30	0.25

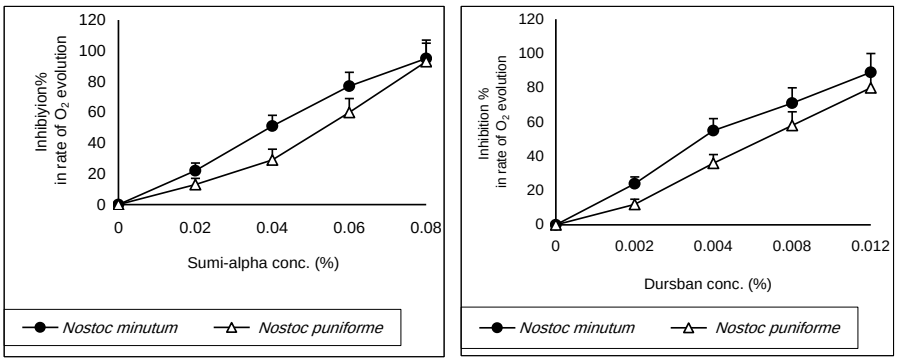


Figure (5). The inhibition % in rate of photosynthetic O_2-evolution in *Nostoc minutum* and *Nostoc puniforme* treated by sumi-alpha (A) and dursban (B) within 3 hours.

The present findings indicate an inhibition in the algal pigmentation, which consequently lead to alteration in the metabolism of carbohydrate. The results of carbohydrate content (Table 3) showed a differential response to the different employed insecticides and the time of treatment.

Table (3). Effect of Sumi-alpha and Dursban insecticides treatment on the total soluble sugars content (mg g^{-1} dry weight) of *Nostoc minutum* and *Nostoc puniforme.* [Data were recorded at sharp logarithmic phase (8 days) and the mean ± SD of three replicates].

Insecticide concentration (%)		Total soluble sugars (mg g^{-1} dry weight).	
		Nostoc minutum	*Nostoc puniforme*
0.0		30 ± 0.2	41 ± 0.02
Sumi-alpha	0.02	32 ± 0.1	36 ± 0.30
	0.04	29 ± 0.3	25 ± 0.50
	0.06	12 ± 0.4	13 ± 0.22
	0.08	8.20 ± 0.1	6 ± 0.40
Dursban	0.002	26.30 ± 0.2	30 ± 0.35
	0.004	20.08 ± 0.3	23 ± 0.22
	0.008	9.20 ± 0.1	8 ± 0.15
	0.012	6.01 ± 0.3	6 ± 0.22
LSD between rows at 5%		18	20

It can be noticed that there was a sharp decrease in total soluble sugars content after treatment with insecticides and the results clearly indicated that the total soluble carbohydrates were generally decreased with increasing the insecticide concentrations. The highest diminution (73, 85, 72 and 85 %) was found for both insecticides (sumi-alpha and dursban) after 8 days of treatment. Similarly, total soluble sugars of both studied algae were reduced by 72 and 85 % at high concentration of dursban (0.012 %) as compared to control after 8 days of treatment.

The changes in the contents of carbohydrate of the studied algae may attributed to the interference of insecticides in the mechanism of their metabolism. Moreover, the changes in the hydrolysis of the more complicated particles to simplest ones and/or the building up of complexes might be also affected (Singh, *et. al.*, 1983,Ghosh and Saha, 1988 and Khalifa-Neveen, 1999) .

The decreased in total soluble sugar contents in the present results paroled with the decreases in the photosynthetic pigments. This indicates a decline in the rate of photosynthesis and suggests that the decrease in sugars could be attributed to an inhibition in the rate of synthesis. In this connection, Khalifa-Neveen, (1999), and Ibraheem, (2002) reported that, application of insecticides such as cypermethrin, fenvalerate, diazinon, cyanophos, larvin and seven were inhibit carbohydrate fractions of *Anacystis nidulans, Scenedesmus bijugatus, Anabaena variablis and A. subtropica.*

The nitrogenase activity of the control and the cyanobacteria treated with different concentrations of the two studied insecticides was determined after 8

days incubation period. All concentrations used of sumi-alpha decreased the N_2-activities of both *Nostoc minutum* and *N. puniforme* as compared to the control (Fig. 6). At concentration (0.08 %) of sumi-alpha the nitrogenase decreased to 10 & zero µL $C_2H_4L^{-1}$ medium hr^{-1} of both *Nostoc minutum* and *N. puniforme* , respectively as compared to the control. From the results obtained in Figure (6), it was found that concentration 0.008 % dursban was the most toxic one and severely affected the nitrogenase activity of both treated cyanobacteria.

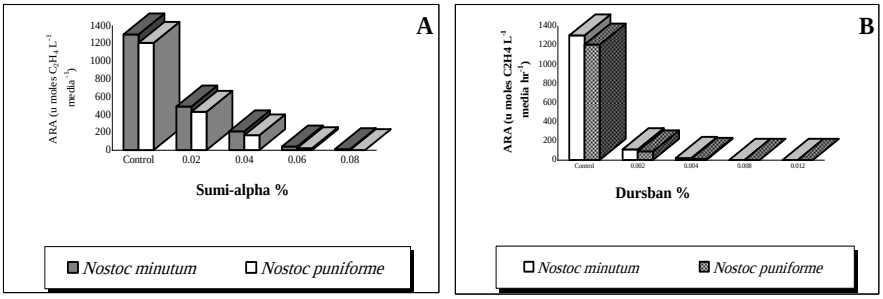


Fig.6. Nitrogenase activity of *Nostoc minutum* and *Nostoc puniforme* treated by sumi-alpha (A) and dursban (B). [(Data were recorded at sharp logarithmic phase (8 days)].

The inhibition of nitrogenase activity was strongly combined with injurious effects on heterocysts. Fay, *et al.,* (1968) reported that heterocysts are the sites of nitrogen fixation. The results reported by many investigators (Singh, 1973, Batterton, *et al.,* 1971, Allen and Arnon, 1955 and Chang, 1979) indicated that inhibition of heterocyst formation by some inhibitory substances, were not always coincident with similar effects on growth, since the later process was increased, at the time which heterocyst formation was ceased. However, several investigators reported a strong relationship between growth and nitrogen fixation (Stewart, *et al.,* 1971 and Bottomely & Stewart, 1977). Fogg & Than-Tun (1960) and Kobbia & El-Sharouny (1983) reported that there is a close connection between photosynthesis and nitrogen fixation, they found that the rates of the two processes were closely correlated under a wide range of growth conditions. The nitrogen fixation process was also reported to depend mainly upon photosynthesis, the carbon skeleton, ATP and hydrogen donor (Wolk, 1968).

It may be suggested that the inhibition of N_2-ase activity by dursban and sumi-alpha applications in two species of *Nostoc* may be attributed to the effect of insecticides on the size of the pool of reductant, which the photosynthetic products provide. It may alter the ratios between CO_2 and O_2 during photosynthesis and photorespiration (Kobbia and El-Sharuny, 1983).

Table 4 shows the effect of sumi-alpha and dursban on the total soluble proteins of *Nostoc minutum* and *N. puniforme*. It was found that the highest inhibition percentages (79 and 85 %) of the total soluble proteins were recorded with treatment by 0.08 % sumi-alpha, respectively. In addition, the obtained data indicated also that the total soluble proteins of both *Nostoc* species were dramatically inhibited by 86-91 % at 0.012 % of dursban, respectively.

Table (4). Effect of the Sumi-alpha and Dursban insecticides on the total soluble protein contents (mg g^{-1} dry weight) of *Nostoc minutum* and *Nostoc puniforme*. [Data were recorded at sharp logarithmic phase (8 days) and the mean ± SD of three replicates].

Insecticide concentration %		Total soluble proteins (mg g^{-1} dry weight).	
		Nostoc minutum	*Nostoc puniforme*
0.0		175 ± 1.12	145 ± 2.33
Sumi-alpha	0.02	139 ± 3.62	122 ± 2.20
	0.04	115 ± 2.25	86 ± 2.40
	0.06	74 ± 1.67	60 ± 0.70
	0.08	36 ± 1.91	22 ± 0.88
Dursban	0.002	137 ± 3.02	111 ± 3.3
	0.004	101 ± 2.71	75 ± 2.6
	0.008	63 ± 1.35	43 ± 2.2
	0.012	25 ± 3.20	13 ± 1.1
LSD between rows at 5%		78	68

Inhibition of protein synthesis caused by insecticides may due to the change in cell membrane permeability which disturbs the absorption and retention of the amino acids available for protein synthesis, interference with the incorporation of amino acids into protein, and/or the formation of enzymes responsible for protein synthesis and metabolism (Egli, *et al.,* 1985). Moreover, the inhibitory effect of the investigated insecticides on the total soluble proteins may be attributed to some of their action as Hill reaction inhibitors which resulted in reduction of ATP. This product is responsible for the major biosynthetic pathways involved in the synthesis of nucleotides amino acids and protein (Monapatra & Mohanty, 1993 and Khalifa-Neveen, 1999). In accordance, Ibraheem, (2002) also observed a decrease in protein content in *Anabaena* sp. treated with larvin and sevin insecticides.

The data in Table 5 showed a marked ability of the two investigated cyanobacteria to stimulate the total soluble lipids by 45 & 55 % at 0.08 % of

sumi-alpha, respectively. As apparent also from Table 5, the values of total soluble lipids of both *Nostoc* spp. treated by dursban recorded a gradual stimulation with increase in dursban concentration. Accordingly, at 0.012 % of dursban the stimulation of the total lipids of *Nostoc minutum*. and *N. puniforme* were 7.3 & 10.8 %, respectively.

Table (5). Effect of Sumi-alpha and Dursban insecticides on the total lipid contents (µg mg^{-1} dry weight) of *Nostoc minutum* and *Nostoc puniforme*. [Data were recorded at sharp logarithmic phase (8 days) and the mean ± SD of three replicates].

Insecticide concentration %		Total lipids (µg mg^{-1} dry weight)	
		Nostoc minutum	*Nostoc puniforme*
0.0		56.44 ± 2.30	60.51± 3.20
Sumi-alpha	0.02	56.32 ± 6.00	62.02 ± 3.30
	0.04	60.56 ± 5.01	70.00 ± 1.10
	0.06	80.74 ± 4.11	93.00 ± 2.40
	0.08	81.82 ± 2.03	94.00 ± 1.50
Dursban	0.002	55.50 ± 2.22	60.02 ± 1.20
	0.004	56.63 ± 4.31	63.30 ± 3.00
	0.008	59.78 ± 3.40	67.05 ± 2.00
	0.012	60.61 ± 5.76	67.06 ± 2.50
LSD between rows at 5%		75	NS

NS non significance

Although lipid levels represent a non-specific indicator of the relative health of an organism, these macromolecules have particular significance when assessing hydrophobic bioaccumulative contaminants. Lipids are essential, readily available energy source, particularly for many photoplankton, and are periodically mobilized from endogenous pools. As a result, bound lipophilic toxins can inturn be mobilized to exert adverse effects on lipophilic membranes such as the plasmmalemma and thylokoids which are particularly sensitive to the effects of hydrocarbons, and in view of the fact toxicants can partition into membranes or adsorb onto them (Kent and Caux, 1995).

The destabilizing effect of sumi-alpha and dursban on membrane structure and permeability may also account for the apparent arrest of cell division in exposed cells. It is not surprising to observe the effects of the two studied insecticides on fatty acids of the treated algae that suggest alterations in membrane structure and permeability. Also stimulation effect of lipid may be due to defense mechanism of the organism to balance this toxic effect (Fisher, 1977). Also, associated fatty acids may influence an algal cells ability to tolerate xenobiotics (Shifrin and Chisholm, 1981).

The result depicted in Table 6 revealed that the fatty acid contents of the total lipids of *Nostoc minutum* decreased with increasing sumi-alpha concentration except hexadecadienoic 16:2 and hexadecatrienoic 16:3 which also had high values (55.7 – 27 %) at 0.08 % of sumi-alpha, respectively. Table 6, revealed that there was obvious inhibition in the fatty acid contents of the total lipids of *Nostoc minutum*. The saturated palmitic acid (16:0) highly reduced to 56% at 0.08 % of sumi-alpha. Furthermore, the unsaturated C_{16} fatty acids estimated as palmitoleic acid (16:1) showed also a remarkable inhibition (53 %) less than control. Sumi-alpha caused also severe inhibition in unsaturated oleic acid (18:1), diunsaturated linoleic (18:2) and polyunsaturated linolenic (18:3) acids which recorded high suppression ratios in the order of 42.8, 58 & 36.4 % , respectively at 0.08 % of sumi-alpha compared with the control.

Table (6). Effect of Sumi-alpha insecticides on the fatty acids contents (mol %) of the total lipids of *Nostoc minutum* cells. [Data were recorded at sharp logarithmic phase (8 days)].

Fatty acids mol %	Sumi-alpha concentration (%)				
	Control	0.02	0.04	0.06	0.08
16:0 Palmitic	21.30	21.33	18.3	12.00	9.3
16:1 almitoleic	4.67	4.81	4.00	3.01	2.20
16:2 Hexadecadienoic	6.41	6.99	7.06	8.67	9.98
16:3 Hexadecatrienoic	9.52	10.00	10.02	11.11	12.10
18:1 Oleic	26.99	26.90	20.00	18.77	15.36
18:2 Linoleic	14.41	15.40	10.10	8.03	6.01
18:3 Linolenic	15.90	15.98	13.35	12.97	10.11

Conclusion

Insecticides under investigation are already known with their interference with the algae metabolism. Chlorpyrephos (Dursban) and S-Fenvalerate (sumi-alpha) are inhibitors of photosynthesis, affecting the integrity of membranes, destroying chlorophyll *a* , carotenoids, blocking the biosynthesis of total soluble carbohydrates, proteins, nitrogenase activity, lipids and fatty acids.

References.

Abdel-Hafez, A.; Moawad, G.M.; EL-Gemeiy; H. M. and Rashad, A. M. (1996). Effect of some insecticides on *Trichogramma evanescens westwood, Trichogrammatoidea bactrae nagaraja,* and the hatchability of *Pectinophora Gossypiella* (Saund.) eggs. *Egyptian Journal of Biological Pest Control,* **6: 1-5**.

Allen, M. B. and Arnon, D. I. (1955). Studies on nitrogen-fixing blue-green algae, I. Growth and nitrogen fixation by *Anabaena cylindrica* Lemm. *Plant Physiol*., **30: 366-372**.

Allen, M. M. (1968.) Simple conditions for growth of unicellular blue-green algae on plates. *J.Phycol*., **4: 1-4**.

Ashton,F.M. and Crafts,A.S.(1981). Mode of action of herbicides. 2nd Ed. Wiley. New York , pp 525.

Awad, E. I. (1978). Effect of some pollutants on some fresh water planktonic organisms. Ph.D. Thesis, Fac. of Agriculture, Cairo University. Egypt.

Bartels, P. and Watson, C. (1978). Inhibition of carotenoid synthesis by fluridone and norflurazon. *Weed Sci*., **26: 198-203**.

Batterton, J. C.; Boush, G. M. and Matsumura, F. (1971). Growth response of blue-green algae to aldrin, dieldein, edrin and other imetabolites. *Bull. Environ. Contam. Toxicol*., **6:589-594**.

Bligh, E. G. and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol*., **37: 911-917**.

Bottomley, P. J. and Stewart, W. D. P. (1977). ATP and nitrogenase activity in nitrogen fixing heterocystous blue-green algae. *New Phytol*., **79: 625-638**.

Campbell, W. R.; Evans, S. O. and Reed, S. C. (1976). Effects of glyphosate on chloroplast ultrastructure of quick grass mesophyll cells. *Weed Sci*., **24: 22-25**.

Caux, P. Y. (1989). The effect and mode of action of Dowanol and a series of non-ionic Triton adjuvants on Lemma minor L. Ph.D. Thesis Dept. of Biology, Univ. of Ottawa, Ottawa, Ont.

Chang, T. P. (1979). Growth and acetylene reduction by *Gloeotrichia echinulata* (Smith) Richter in axenic culture Br. *Phycol. J*., **14: 207-210**.

Duke, S. O.; Lydon, J.; Becerril, J. M.; Sherman, T. D.; Lehnen, L. P. and Matsumoto, H. (1991). Protoporphyrinogen oxidease-inhibiting herbicides. *Weed Sci*., **39: 465-473**.

Egli, M. A.; Low, D.; White, K. R. and Howard, J. A. (1985). Effect of herbicides and herbicide anologe on (^{14}C) leucine incorporation by suspension cultured *Solanum nigrum* cells. *Pestic. Biochem-Physiol*., **24: 112-118**.

Fay, P.; Stewart, W. D. F.; Walsby, A. E. and Fogg, G. E. (1968). Is the heterocyst the site of nitrogen fixation in blue-green algae . *Nature, Lond*., **220: 810-812**.

Fisher, N. S. (1977) On the differential sensitivity of estuarine and open ocean diatoms to exotic chemical stress. *Am. Nat*., **111:871-895**.

Fogg, G. E. S. and Than-Tun (1960). Interrelations of photosynthesis and assimilation of elementary nitrogen in a blue-green algae. *Proc. R. SOC. Lond. B.*, **163:111-127**.

Grenier, G.; Marier, J. P. and Beaumont, G. (1979). Effects physiologiques de l' atrazine à doses sublétales sur *Lemna minor* L. IV. Influence sur la compósition lipidique. *Can. J. Bot*., **57:1015-1020**.

Ghosh, T. K. and Saha, K.C. (1988). Influence of carbofuran on the growth and nitrogen accretion by blue-green algae (cyanobacteria), *Aulosira fertilissima*. *India Agric*., **32 (3): 153-161**.

Handel, E. V. (1968). Direct microdetermination of sucrose. *Anal. Biochem*., **22: 280-283**.

Hughe's, E. O.; Gorham, P.R. and Zehnder, A. (1958). Toxicity of a unialgal culture of *Microcystis aeruginosa*. *Can. J. Microbiol*., **4: 225-236**.

Ibraheem, I. B. M. (2002). Hazard application of two common insecticides (Larvin and Sevin) on the growth, metabolic activity and nitrogen fixing capacity of Cyanobacteria. *Egyptian J. of Phycol.*, **3: 104-115**.

Kates, M. (1972). Techniques of lipidology: isolation, analysis and identification of lipids. In laboratory techniques in biochemistry and molecular biology. Vol. 3. Edited by T.S.Work and E. Work. Elsevier Science Publishing Co., Inc., New York., pp.393-465.

Kent, R.A. and Caux, P.Y. (1995). Sublethal effects of the insecticide fenitrothion on freshwater phytoplankton. *Can. J. Bot*., **73: 45-53**.

Khalifa-Neveen, A. M. (1999). Assessment of the impact of environmental stresses on algae and their effect on the algal cellular macromolecules. Ph.D. Thesis, Fac. Sci., Cairo Univ., Beni-Suef Branch, Beni-Suef, Egypt.

Kobbia, I. A. and El-Sharouny, H. M. (1983). The effect of 2,4-D on nitrogen fixing capacity of some blue-green algae. Egyptian Society of Applied Microbiology Proc. V Conf. Microbiol., Cairo, May 1983. Vol. II-Soils & Water Microbiol. Paper No. 50.

Kochert,G. (1978). Quantitation of the macromolecular components of microalgae. In J. A. Hellebust and J.S. Craigie, eds., Handbook of Phycological Methods: Physiological and Biochemical Methods. Cambridge Unive. Press, Cambridge, UK, pp.189-195.

Krinsky, N. I. (1967). The role of carotenoid pigments as protective agents against photosynthesized oxidation in chloroplasts In: Biochemistry of chloroplast. (Goodwine, T. W. ed.), Vol. I, Acadimic Press, New York, pp. 421-430.

Kunert, K. J. and Boger, P. (1981). The bleaching effect of the diphenylether oxyfluorfen. *Weed Sci*., **29: 169-173**.

Lowery, O. H.; Rosebrought, N.J.; Furr, A. and Randall, R. J. (1951). Protein measurement with folin phenol reagent . *J. Biol. Chem*., **193 : 265-275**.

Metcalfe, L. D.; Schmitz, A. A., and Pelka, J. R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal. Chem*., **38: 514-515**.

Metzener, H.; Rav, H. and Senger, H. (1965). Untersuchungen Zur Synchronisier-barkeit einzelner pigment. Mangol-mutanten von *Chlorella*. *Planta*, **65: 186-194**.

Monapatra, P.K. and Mohanty, R.C. (1993). Comparative toxicity of two pesticides to a green alga *Chlorella vulgaris* . *Acta Botanica Hungarica*., **38 (1-4): 257-272**.

Nikolenko, A. G. and Amirkhanov, D.V. (1993). Comparative hazardousness of various types of insecticides to soil algae. *Eurasian Soil Science*, **25(8): 103-110**.

Ono, T. A. and Murata, N.(1981). Chilling susceptibility of the blue-green alga *Anacystis nidulans*. *Plant Physiology*, **67: 176-181**.

Prescott, G .W. (1951). Algae of the Western Green Lakes area. Exclusive of desmids and diatoms. Bull. No. 31, Cranbrook Inst. of Sci.

Prescott, G. W. (1969). The algae. A review. Michigan State Univ., Butler & Tanner Ltd, Frome and London.

Prescott, G .W. (1978). How to know the freshwater algae. 3rd Ed. Wm. C. Brown Company Publishers. USA.

Ricotta, J. A. and Masiunas, J. B. (1992). Acifluorfen tolerance in *Lycopersicon*. *Weed Sci*., **40: 413-417**.

Roger, P. A.; Ardales, T. S. and Watanabe, I. (1986). Chemical composition of cultures and natural samples of N_2-fixing blue-green algae from rice fields. *Biol Fertil Soils*., **7: 131-146**.

Shifrin, **N. S. and Chisholm, S. (1981)**. Phytoplankton lipids : Interspecific differences and effects of nitrate, silicate and light dark cycles. *J. Phycol*., **17 : 374 – 384**.

Sicko-Goad, L.; Hall, J.; Lazinski, D. and Simmons, M. S. (1989). Effects of chlorinated benzenes on diatom fatty acid composition and quantitative morphology. II. 1,3,5-trichlorobenzene. *Arch. Environ. Contam. Toxicol*., **18: 629-637**.

Singh, P. K. (1973). Effect of pesticides on blue-green algae. *Arch. Mikrobiol*., **89: 317-320**.

Singh, P. K. (1974). Algicidal effect of 2,4-D on blue-green alga *Cylindrospermum* sp. *Arch. Mikrobiol*., **97: 73-80**.

Singh, R. K.; Singh, B. D. and Singh, H. N. (1983). Inhibition of photosystem II of nitrogen-fixing blue-green alga *Nostoc linckia* by the rice- field herbicide benthiocarb. Z. Allg. *Mikrobiol*., **23: 435-441**.

Stewart, W. D.P.; Fitzgerald, G.P. and Burris, R.H. (1971). In situ studies on N_2 – fixation using the acetylene reduction technique. *Proc. Natn. Acad. Sci. U.S.A.*, **58:2071-2078**.

Wolk, C. P. (1968). Movement of carbon from vegetative cells to heterocysts in *Anabaena cylindrica. J. Bact*., **96: 2138-2143**.

Yemm, E. W. and Willis, A. J. (1954). The estimation of carbohydrates by anthrone . *Biochem. J.*, **57: 508-514**.

Zar, J. H. (1984). Biostatistical analysis. 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, N.J.

التأثير الضار لمبيدين من المبيدات الحشرية (سيمي الفا و دورسبان) علي النمو والنشاط الأيضى لنوعين من جنس النوستك المثبتة لنيتروجين الهواء الجوي.

نيفين عبد الرؤوف

قسم النبات - كلية العلوم - جامعة القاهرة - بني سويف - مصر .

تم في هذه الدراسة تقدير تأثير الخطر البيئي الواقع علي نوعين من النوستك من جراء استخدام التركيزات العالية من أثنين من المبيدات الحشرية التي تستخدم في التربة المصرية بنسبة عالية وهما (سيمي الفا ودورسبان) ومدى الخطورة علي كفاءة هذه الأنواع من السيانوبكتيريا في تثبيت نيتروجين الهواء الجوي وكذلك تأثير هذه المبيدات علي البناء الحيوي داخل خلاياها.

أكدت النتائج أن التركيزات المستخدمة في هذا البحث لهذين المبيدين (0.02 – 0.08 % من سيمي الفا ، 002. – 012. % من دورسبان) والتي أقل بكثير من التركيزات المستخدمة في الحقول المصرية (0.1 % من سيمي الفا ، 0.17 % من دورسبان) لها تأثير ضار علي المحتوي الكلي لكلوروفيل (أ) والكاروتينات والكربوهيدرات والوزن الجاف ونسبة الأكسيجين المتصاعد للطحالب وكذلك أدت هذه التركيزات المختلفة من كلا المبيدين إلى انخفاض عملية تثبيت النيتروجين والمحتوي الكلي للبروتين الذائب وكذلك الأحماض الدهنية الليبيدية ،إلا أن الدهون الكلية أظهرت زيادة ملحوظة عند معظم المعاملات المستخدمة.