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HAZARD APPLICATION OF TWO COMMON INSECTICIDES (LARVIN AND SEVIN) ON THE GROWTH , METABOLIC ACTIVITY AND NITROGEN FIXING CAPACITY OF CYANOBACTERIA.

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Abstract

Two soil cyanobacterial species were used to investigate the factors affecting the toxicity of two common used insecticides, larvin (Dimethyi N,N-[thiobis (methyl amino carbonyloxy)]-bis-(ethanimidothioate) and sevin (1-Naphthyl N-methylcarbamate) to soil microflora. The heterocystic filamentous *Anabaena subtropica* Gardner and *Anabaena variablis Kützing* ex.Born. *et* Flah., were exposed to four concentrations (5,15,20 and 40 mg L^{-1}) of larvin and sevin. Differential growth effects were observed among the two cyanobacterial species exposed to all insecticides concentrations. Selected properties of the test cyanobacteria (chl *a*, dry weight, O_2 evolution, nitrogenase activity, total soluble proteins, total lipids and fatty acid contents of the total lipids) were measured and examined for their relationship to larvin and sevin sensitivity. Strong inhibiting percentages in all measured parameters in both *Anabaena* species under the different pesticides concentrations of larvin and sevin except the total lipids were recorded, suggesting that these attributes to the potential hazard of these insecticides to soil microflora and subsequently natural ecosystems.

Key words: *Anabaena* Larvin Sevin Hazard evaluation.

Introduction.

Soil is a dynamic system in which the physical, chemical and biotic components are in a state of equilibrium. Application of insecticides without taking care of the other soil constituents, disturb this equilibrium which adversely affects the productivity of the soil. Maintenance of the soil biota other than the harmful pests help in better crop nutrient management and maintenance of soil health while working in perfect harmony with the nature. Insecticides frequently exert inhibitory or stimulatory effects on the growth or other activities of microorganisms, either in pure culture or in the field. Few works on pesticides distributions, types, toxicity, mechanism of actions, degradations, their tolerance by the organisms and other phsiological processes were reviewed and summarised. Ramachandran *et al.,* (1980) and Ghosh & Saha (1988) suggesting that some pesticides actually are highly phytotoxic, such as carbaryl. The toxicity of carbaryl was studied also by Peterson *et al.,* (1994) and Fletcher, (1990). Blue-green algae , specially the nitrogen-fixers cyanobacteria represents 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). Also, cyanobacteria have been assumed to produce growth promoting substances like hormones, vitamins, amino acids or many other components that enhances germination (Singh & Trehan, 1973; Grieco and Desrochers, 1978; de Carire *et al.,* 1997; Rodgers *et al.,* 1979; Venkataraman, 1981; de Mule *et al.,* 1999; Godd *et al.,* 1999 and Omar, 200). Most of the soil and aquatic microscopic algae are sensitive to insecticides due to the fact that algae are engaged in photosynthesis and that many insecticides interfere with the process. It is of particular

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interest to mention that algae participate activity in the binding and neutralizing of xanobiotics (Jampani, 1989 and Baeza-squiban *et al.*, 1990).

In Egypt , larvin (Thiodicarb) and sevin (Carbaryl) are the major applied insecticides to get rid of bollworms and other cotton pests. Higher concentrations of these pesticides 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 present study was conducted to evaluate the hazard presented by these insecticides to the non target organisms specially cyanobacteria which concidered as a major component of soil contents. The insecticides tested were selected on the basis of recommendations of Ministry of Agriculture and land Reclamation, for the integrated control of cotton bollworms. The common and trade names, and the prescribed field applications rates are given in Table (1).

Table (1). Details of insecticides used in the experiment.

Common name	Trade name	Chemical name	Dosage*/ Fadan
Carbaryl	Sevin	1-Naphthyl N-methylcarbamate.	1500 gm
Thiodicarb	Larvin	Dimethyi N, N- [thiobis (methyl amino carbonyloxy)]- bis-(ethanimidothioate).	460 gm

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

Materials and Methods.

Cyanobacterial isolates. Two cyanobacterial species were isolated from cotton fields . *Anabaena subtropica* Gardner and *Anabaena variablis Kützing* 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 pure isolates of algae were inoculated in 100 ml sterilized liquid Allen's 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 of 3000 Lux through light-dark cycle of 16-8 hrs respectively during the growth time, with optimum growth time 10 days.

Chemicals tested. Two common used insecticides, larvin (Dimethyi N,N-[thiobis (methyl amino carbonyloxy)]-bis-(ethanimidothioate) and sevin (1-Naphthyl N-methylcarbamate) (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 5,15,20 & 40 mg L^{-1}.

Measurement of treated algal growth.

Chlorophyll *a* estimation. Chlorophyll *a* was estimated by the method of Strickland and Person (19972).

Dry weight determination. At the end of 10 days (logarethmic growth phase) the culture masses were separated from their media by suction filtration then dried at 90 °C many times till a constant weight was attained.

Oxygen evolution determination. Photosynthetic oxygen evolution was polarigraphically determined by following the changes in O_2 concentrations 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 $0.1 mg\,ml^{-1}$, were placed in temperature controlled cuvette and illuminated with a quantum flux density of 300 $\mu Em^{-2} s^{-1}$.

The Metabolic analyses.

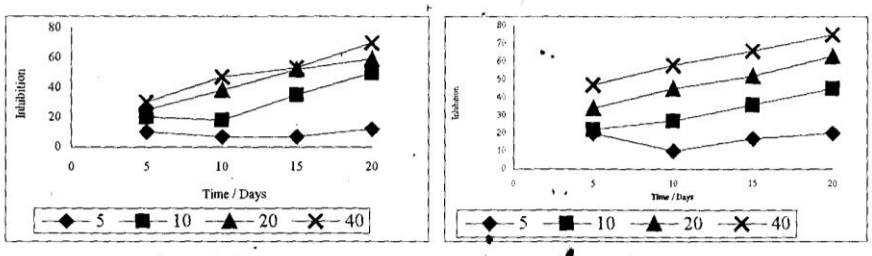
All the following parameters were estimated at sharp logarethmeic phase (10 days old culture)

1. **Measurement of Nitrogenase activity (acetylene reduction method).** The cultures were grown without nitrogen source for estimation of the nitrogen fixation ability of the two studied cyanobacteria using acetylene reduction technique of Stewart *et al.*,(19971).
2. **Total soluble proteins**. This was carried out according to the method of Lowery *et al.,* (1951), using bovine serum albumin as a standared protein.
3. **Total lipid content**. A quantitative determination of total cellular lipids using dichromate reduction method (Kochert's, 1978) was conducted.
4. **Fatty acid content of total lipids**. The effect of larvin on the composition and quantity of fatty acids of the total lipids were examined in *Anabaena variablisi Kützing* ex.Born. *et* Flah. Cells. Replicate samples were harvested from control and treated cultures and separated from the growth medium by centrifugation. Prior to this step, subsamples were taken for cell enumeration 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 to 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 to chloroform to 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 (botton 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.

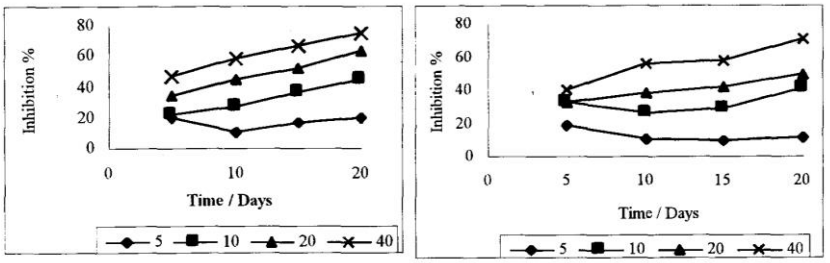
Results and discussion

Growth of *Anabaena subtropica* Gardner and *Anabaena variablis Kützing* ex.Born. *et* Flah as monitored by estimation of chl *a* and dry weight contents was inhibited by addition of larvin and sevin to the cultures. The data recorded in Fig. 1 showed the effect of larvin and sevin on chl. *a* . content of cyanobacterium *Anabaena subtropica* Gardner. It is clear that a progressive inhibition in percentages of hl. *a* were bserved with ncreasing of larvin & sevin concentrations. Where, the highest inhibition percentages of chl. *a* (70 & 74 %) of the studied cyanobacterium were recorded at concentration of 40 mg L^{-1} for both larvin and sevin respectively after 20 days of treatment. Similarly, the data given in Fig (2) indicated that. the treatment of *Anabaena variablis Kützing* ex.Born. *et* Flah. with low concentration of both larvin and sevin (5mg L^{-1}) caused a moderately depression in its chl. *a* biosynthesis (20 & 11 %) after 20 days of treatment, respectively. Further increase of larvin concentrations (15 & 20 mg L^{-1}) highly suppressed chl. *a* values in treated *Anabaena variablis Kützing* ex. Born. *et* Flah to 45 & 63 % after 20 days respectively. Also, application of 10 & 20 mg L^{-1} of sevin in the nutrient medium of *Anabaena variablis Kützing* ex.Born. *et* Flah. led to a gradual inhibition in chl. *a* contents (41 & 49 %) after 20 days, respectively comparable with corresponding control. Furthermore, the inhibition percentages of chl.*a* linearly increased (75 & 71 %) with increasing of larvin and sevin concentrations (40 mg L^{-1}) respectively. These results are in harmony with findings of Shabana (1991). She found that, growth, dry weight and the total sluble carbohydrates of *Anabaena oryzae* and *Aulosira fertillssima* were significantly decreased by application of Parathion pesticide. Also, similar results were previously recorded by Singh, *et al.*, (1983). They reported that, Thiocarbamate benthiocarb was inhibiting for of growth of *Nostoc linckia.* In addition, they recorded a reduction in heterocyst formation at all studied insecticides concentrations it was found that, the heterocyst differentiation in both studied cyanobacteria is completely ceased.



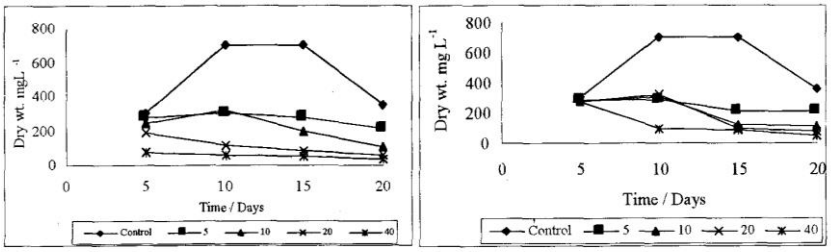
(A) (B)

Fig.1. Inhibition percentage of chl *a* contents of *Anabaena subtropica* Gardner treated by different conce. of Larvin (5-40 mgL^{-1}) (A) and Sevin (5-40 mgL^{-1}(B).



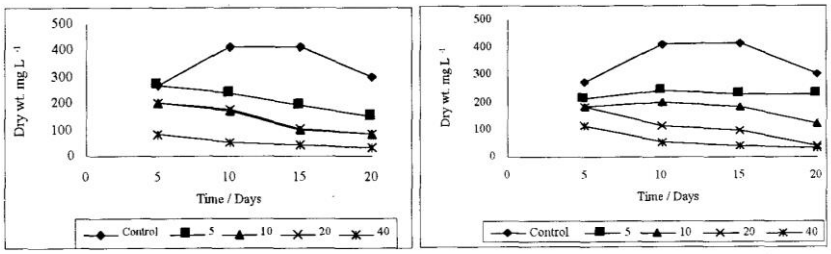
(A) (B)

Fig.2. Inhibition percentage of chl *a* contents of *Anabaena variablis Kützing* ex. Born. *et* Flah treated by different conc. of larvin (5-40 mgL^{-1}) (A) and Sevin (5-40 mgL^{-1}) (B).



(A) (B)

Fig.3.Dry weight contents of *Anabaena subtropica* Gardner treated by different conc. of larvin (5-40 mgL^{-1}) (A) and sevin (5-40 mgL^{-1}) (B).



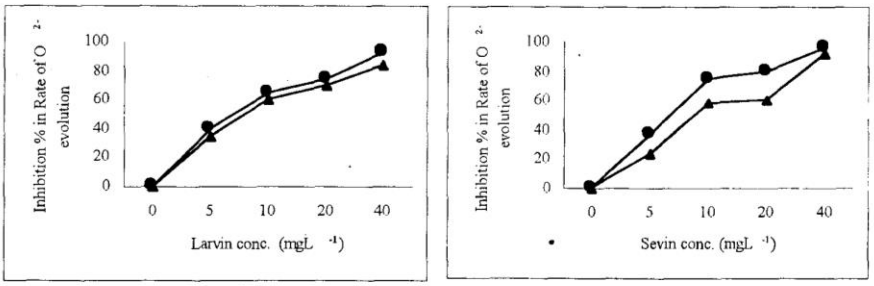
(A) (B)

Fig.4. Dry weight contents of *Anabaena variablis Kützing* ex. Born. *et* Flah treated by different conc. of larvin (5-40 mgL^{-1}) (A) and sevin (5-40 mgL^{-1}) (B).

A gradual inhibitions of dry weight of *Anabaena subtropica* Gardner were observed with increasing of the two studied insecticides (Fig. 3). Accordingly, increasing larvin and sevin concentrations to 5, 10 & 20 mg L^{-1} caused high reduction in (70, 84 &

92 %) and (70, 84 & 90 %), respectively comparable with the control. In addition the results given in Fig. 4 revealed that 40 mgL^{-1} of both larvin & sevin caused high inhibition of dry weight of the treated *Anabaena variablis Kützing* ex.Born. *et* Flah to 93 & 92.7 %, respectively, after 20 days comparing with controls. Successive concentrations of larvin & sevin (5, 10 & 20 mg L^{-1}) recorded a drastic inhibition ratios (63.6, 80.6 & 80 %) and (44, 71 & 90.3 %) in dry weights of the cyanobacterium *Anabaena variablis Kützing* ex.Born. *et* Flah. Respectively. (Fig.4). In this respect, Ramachandran *et al.*, (1980) reported that, the growth of the marine diatom, *Coscinodiscus concinnus* was inhibited by 46% when exposed to only 0.05 mg L^{-1} carbaryl. They suggested that carbaryl may actually be highly phytotoxic to certain plants (Ghosh & Saha, 1988).

The interference of larvin and sevin with growth and chl *a* was further clarified by testing the insecticides effects at different concentrations on the photosynthetic electron flow (Fig. 5). The photosynthetic activity in both *Anabaena* species measured as O_2 evolution showed marked inhibition at all insecticides concentrations used (5,10,20 & 40 mg L^{-1}).



(A) (B)

Fig.5. The rate of photosynthetic O_2-evolution in *Anabaena subtropica* Gardner (-•-) and *Anabaena variablis Kützing* ex. Born. *et* Flah. (-▲-) treated by different conc. of larvin (5-40 mgL^{-1}) (A) and sevin (5-40 mgL^{-1}) (B).

The reduction in O_2 evolution rate reached approximatly 60 and 65% in *Anabaena subtropica* Gardner and *Anabaena variablis Kützing* ex. Born. *et* Flah at 10 mg L^{-1} of larvin and 58 and 75% in *Anabaena subtropica* Gardner and *Anabaena variablis Kützing* ex. Born. *et* Flah at 10 mg L^{-1} of sevin, respectively. Where the inhibition percentages were progressively increased with increasing of insecticides concentration in all treatments.

The results showed more or less similar pattern of change in a photosynthetic response to O_2 evolution as in growth in response to insecticides treatment. Singh *et al.*, (1983) showed that low concentrations of benthiocarb induced a reduction in oxygen evolution in *Nostoc linckia.*

Nitrogen fixation by cyanobacteria, is an important source of nitrogen input in the nitrogen cycle of cultivated soils and could limit pollution problems by lowering the demand for chemical fertilizers (Quesada *et al.*, 1997). Nitrogenase activities in both experimental cyanobacteria were found to be affected by both larvin and sevin. These insecticides inhibit the enzyme action, not only at higher doses, but also on the lower doses (Fig. 6 A and B). A gradual suppresion in nitrogenase activity by increasing of larvin concentration could be observed. The obtained results indicated that increasing of

sevin concentration (5, 10 & 20 mg L^{-1}) led to significant inhibitions of nitrogenase activity values of *Anabaena subtropica* Gardner (Fig. 6-A) and of *Anabaena variablis Kützing* ex. Born. *et* Flah. (Fig. 6-B).

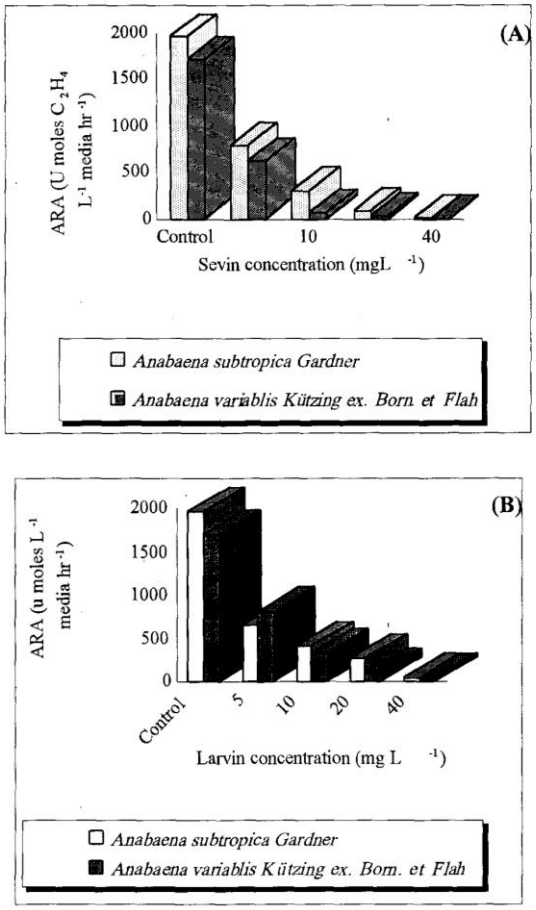


Fig.6. Nitrogenase activity of *Anabaena subtropica* Gardner. and *Anabaena variablis Kützing* ex. Born. *et* Flah affected by larvin (A) and sevin (B)treatments. (Data were recorded at sharp logarithmic phase).

The nitrogen fixation process depends mainly on an equilibrium between CO_2 & O_2 gases. This ratio is specific but generally under high O_2 – tension inhibition of acetylene reduction occurred (Stewart & Pearson, 1970). These ratios of CO_2 / O_2 are controlled by respiration and photosynthesis processes (Lex *et al.*, 1972). The dependence of nitrogen fixation process on photosynthesis comprises also, the carbon skelton, ATP

and hydrogen donor (Wolk, 1968). It may be suggested that the inhibition of Nitrogenase-activity by larvin and sevin application in the tested blue green species, as well as, the un-stability for sometimes in the patterns of aerobic & anaerobic inhibitions, may be attributed to the effect of larvin and sevin on the size of the pool of reduction, which the photosynthic products provide. It may alter the ratios between CO_2 & O_2 during photosynthesis and photorespiration. The rate of inhibition and its severity depends on the organisms under test and concentration of the pesticides.

Kobbia and EL-Sharony (1983) reported that, Nitrogenase activity was inhibited in three blue-green algae by treatment with 2,4-D herbicide. In addition, the reduction in nitrogenase activity could be attributed to inhibition in protein synthesis by applied insecticides (Singh, *et al.*, 1983).

Data presented in Table 2 indicated that total soluble proteins were gradually inhibited by increasing larvin and sevin doses. At low dose (5 mg L^{-1}) of larvin and sevin, the total soluble proteins of *Anabaena subtropica* Gardner were reduced by 45.2 and 33 %, respectively as compared with control. Increasing the doses to 10 and 20 mg L^{-1} of larvin and sevin lead to high inhibitory levels in total soluble proteins of *Anabaena subtropica* Gardner to 81.3 & 93.7 % for larvin and 75.4 & 86.2 % for sevin. Morever, at the highest dose (40 mg L^{-1}) of both larvin and sevin, the total soluble proteins were ceased (97.7 & 91.8%) respectively, comparing with the control.

Table 2. Effect of larvin and sevin treatments on total soluble protein contents (mg g^{-1} dry algae) of *Anabaena subtropica* Gardner & *Anabaena variablis Kützing* ex. Born. *et* Flah. (Data were recorded at sharp logarithmic phase).

Insecticide concentration (mgL^{-1})		Total soluble proteins (mg g^{-1} dry algae).	
		Anabaena subtropica Gardner	*Anabaena variablis Kützing* ex. Born. *et* Flah
Control		99.20 ± 3.0	101 ± 2.3
Larvin	5	54.30 ± 3.5	58.8 ± 1.6
	10	18.50 ± 2.0	24.9 ± 2.2
	20	06.21 ± 0.5	17.3 ± 1.3
	40	02.30 ± 0.6	06.5 ± 0.1
Sevin	5	66.50 ± 3.0	73.5± 3.2
	10	24.37 ± 2.2	36.7 ± 1.1
	20	13.60 ± 1.3	20.1 ± 0.5
	40	08.12 ± 1.1	13.4 ± 0.6

Values, expressed on a dry mass basis, and presented as the mean ±SD of three replicates.

Similarly, estimation of the total soluble proteins of *Anabaena variablis Kützing* ex. Born. *et* Flah. after treatments by larvin and sevin (40 mg L^{-1}) showed a pronounced declines representing percentages of 93.6 and 86.7 % (Table 2). Protein synthesis was progressively inhibited by insecticides treatments, a phenomenon which was correlated with the inhibition of nitrogen fixation process (Awad, 1978, Bottomely and Stewart, 1977). These results in agreement with Flectcher, (1990), Ramachandran *et al.*, (1980) and Peterson *et al.*, (1994) who found that, the inhibition in growth of cyanobacteria at low concentrations of some pesticides may be due to their toxic effects or their degradation products on the algal cells. This suggestion was in

agreement with Singh *et al.*, (1983) & Pipe (1992) who classified carbamates as protein synthesis inhibitors and Fletcher (1990) who found that, carbamates have inhibitory effects on the basic metabolic processes common throughout the plant kingdom. Several studies were applied in this respect to estimate the hazard effects of theses chemicals on non target microorganisms.

The all measured parameters were coincided except the total lipids which exhibit stimulating effects with increasing of the studied insecticides concentrations. Data in Table 3 revealed that the total lipid content of *Anabaena subtropica* Gardner were slightly inhibited at low concentrations (5 mg l^{-1}) of the two studied insecticides. However by increasing larvin and sevin concentrations (10, 20 & 40 mgL^{-1}) exhibited a stimulatory effect on total lipid contents of *Anabaena subtropica* Gardner. It reached 36, 77.7, & 77.5 % with treatment by larvin and 19.8, 59 & 61 % with treatment by sevin respectively.

Similarly, the total lipid contents of *Anabaena variablis Kützing* ex. Born. *et* Flah increased with increasing of larvin & sevin concentrations (Table 3). It reached 30.4, 85.5 & 81.3 % with treatment by 10, 20 & 40 mg L^{-1} of larvin and 29, 51.4 & 49.5 % with treatment by 10, 20 & 40 mg L^{-1} of sevin, respectively.

A similar patterns were observed for the total lipid contents of both organisms. treated with larvin and sevin, so, the fatty acids contents of only one of the two studied cyanobacteria (*Anabaena variablis Kützing* ex. Born. *et* Flah.) was determined as the fatty acids of total lipids.

Table 3. Effect of larvin and sevin treatments on total lipids contents (µg mg^{-1} algae) of *Anabaena subtropica* Gardner & *Anabaena variablis Kützing* ex. Born. *et* Flah. (Data were recorded at sharp logarithmic phase).

Insecticide concentration (mgL^{-1})		Total lipid (µg mg^{-1} algae).	
		Anabaena subtropica Gardner	*Anabaena variablis Kützing* ex. Born. *et* Flah
Control		41.4 ± 1.7	30.5 ± 3.3
Larvin	5	40.6 ± 2.3	30.5 ± 3.6
	10	56.4 ± 4.1	39.8 ± 3.2
	20	73.6 ± 5.3	56.6 ± 4.3
	40	73.5 ± 4.5	55.3 ± 5.1
Sevin	5	41.3 ± 2.1	31.60 ± 1.2
	10	49.6 ± 3.0	39.45 ± 2.9
	20	65.9 ± 4.2	46.20 ± 5.7
	40	66.8 ± 2.0	45.61 ± 4.6

Values, expressed on a dry mass basis, and presented as the mean ±SD of three replicates.

Table 4 indicated that increasing in larvin concentrations exhibited an inhibitory effects on estimated fatty acids. The highest reduction values were observed at the high concentrations of larvin (40 mgL^{-1}). Table 4 indicated that the saturated palmitic acid (16:0) slightly affected by larvin treatment (18 mol %) comparing with its control (20.3 mol %). Furthermore, the unsaturated C16 fatty acids which estimated as palmitoleic acid

(16:1) showed a remarkable inhibition (6.67 mol %) less than corresponding control (11.07 mol %).

Table 4. Effect of larvin treatment on fatty acids contents (mol %) of total lipids of the studied cyanobacterium (*Anabaena variablis Kützing* ex. Born. *et* Flah) cells. (Data were recorded at sharp logarithmic phase).

Fatty acids	Larvin concentration (mg L^{-1})				
	Control	5	10	20	40
16:0 Palmitic mol %	20.33	20.92	22.02	20.33	18.17
16:1 Palmitoleic mol %	11.07	11.6	9.90	8.05	6.67
18:0 Stearic mol %	12.52	12.30	7.00	7.55	5.43
18:1 Oleic mol %	20.73	20.00	22.07	19.33	16.41
18:2 Linoleic mol %	5.70	5.03	03.66	3.30	3.00
18:3 Linolenic mol %	30.82	33.63	28.70	27.01	26.23

A gradual inhibitions of saturated stearic acid (18:0) were observed with increasing of larvin concentrations. Accordingly, increasing larvin concentration to 40 mgL^{-1} highly reduced stearic acid (18:0) content to 5.43 mol % comparable with the control (12.5 mol %). Furthermore, larvin inhibited unsaturated oleic acid (18:1) especially at higher concentrations, except at concentration of 10 mg L^{-1} which caused slight increase (22.07 mol %) comparing with the corresponding control (2.02 mol %). Increasing of larvin concentration (40 mg L^{-1}) led to high inhibition in oleic acid content (16.4 mol %).

Also, 40 mg L^{-1} of larvin was highly effective inhibitor in di-unsaturated linoleic (18:2) and polyunsaturated linolenic (18:3) acids, it showed high suppression ratios accounted for 3 & 26.2 mol % comparing with the control (5.7 & 30.8 mol %) respectively. In this respect, the obtained data are in agreement with the findings of Reynolds, (1984), and Kent & Currie, (1995), who found that, many phytoplankton species typically store photosynthetic energy reserves as lipids.

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).

Conclusion.

The shifts in phytoplankton species profiles may be predicated to the contamination which produced from using chemical insecticides (Gaggi, *et al.*, 1995). The recorded results indicated that, both insecticides (larvin and sevin) were applied in Egypt in a high concentrations which considered as a lethal concentrations for the majority of microorganisms such as non target microorganisms and/or the natural predators. The hazard effects of these insecticides not only on this biota but it extend on the crops or

vegetables, which cultivated after cotton harvesting. So, assessment the effects of the studied insecticides on natural ecosystems may requires additional tools that consider environmental factors and/or increase ecological realism. These approaches are still being developed and represent future challenges in ecotoxicology.

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الملخص العربى

الأضرار الناتجة من استخدام المبيدات الحشرية (لارفين ، سيفين) علي النمو والنشاط الأيضى للطحالب الخُضر المزرقة ومدى كفاءتها في تثبيت النيتروجين.

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تم في هذا البحث دراسة مدي الخطر البيئي الناتج من استخدام التركيزات العالية من أثنين من المبيدات الحشرية التي تستخدم في التربة المصرية بنسبة عالية وذلك علي نمو أثنين من الطحالب الخُضر المزرقة (المعزولة من نفس التربة التي يستخدم فيها هذه المبيدات) ومدى كفاءتها الحيوية في تثبيت النيتروجين للتربة وكذلك تأثير هذه المبيدات علي البناء الحيوي داخل خلاياها

أكدت الإحصائيات الناتجة أن التركيزات المستخدمة في هذا البحث لهذين المبيدين (٥ – ٤٠ مليجرام لكل لتر ماء) والتي أقل بكثير من التركيزات المستخدمة في الحقول المصرية (٦٠٠ مليجرام لكل لتر) لها تأثير ضار علي مكونات الطحالب المثبتة لنيتروجين الهواء الجوي. حيث أدت التركيزات المستخدمة في الدراسة إلى درجة تثبيط عالية في المحتوي الكلي لكلوروفيل (أ) 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.

من خلال هذه النتائج نوصي بحذر استخدام التركيزات العالية من هذين المبيدين وكذلك دراسة الخطر الناجم من استخدام التركيزات القليلة منها علي مكونات البيئة الطبيعية ، حيث أنه يتوقع لهذين المبيدين أضرار بيئية مختلفة.