EFFECT OF 2, 4-DICHLOROPHENOXY ACETIC ACID ON CYANOBACTERIA (ANABAENA SP) GROWTH IN THE PRESENCE OF EITHER GLOCUSE OR TRYPTOPHANE

***Skina T. A. Tantawy and ** El Sayeda A. Hassan**

** Botany Department, Faculty of Sience, Cairo University, Giza, Egypt*

***Agricultural Microbiology Department, Soils, Water & Environ. Res. Inst., Agric. Res. Center, Giza, Egypt*

Abstract

2,4-Dichiorophenoxyacetic acid (2,4-D) stimulated growth and heterocyst differentiation of *Anabaena* sp. in nitrogen free medium at low concentration 100 µg mL^{-1} while its higher concentrations inhibited both processes and 500 µg mL^{-1} proved to be lethal. Dry mass and specific growth rate of algae declined with increasing concentration of 2,4-D in the range of 100-1500 µg mL^{-1}. Glucose slightly increased the heterocyst frequency without any lag in their differentiation. Tryptophan promoted growth of the alga and formation of heterocysts (nearly three fold). Tryptophan (50 µg mL^{-1}) complex medium with 1 mg 2, 4-D per ml did not produce mature heterocysts. The filaments were fragmented at the point of heterocyst development and detached heterocyst germinated in situ. Glucose and tryptophan protected the alga, its growth and heterocyst differentiation even at the lethal concentration of the herbicide.

Introduction

The contribution of nitrogen fixing blue-green algae in the nitrogen fixation economy of rice fields is well recognized where they are one of the dominant components of the microflora (Singh, 1961, El-Nawawy *et al*., 1968, Watanabe and Yamamoto, 1971, Daw and Than 1981, Watanabe and Liu 1992, and El-Kholy, 1997). The heterocysts of filamentous nitrogen fixing blue-green algae are thought to function as the site of nitrogen fixation (Fay *et al*., 1968; Stewart *et al*., 1969; Fay, 1973). Studies on the physiology of heterocysts suggested that they have an oxygen protective mechanism for algal nitrogenase under aerobic growth condition (Tel-Or and Stewart, 1977). The extensive use of the herbicides create microbial imbalance causing a great setback to the nitrogen status of soil (Tiwari *et al*., 1981; Pandey, 1985). The magnitude of the herbicide toxicity in plants is govorned by organic carbon, (Singh and Vaishampayan *et al*., 1978), amino acids, Mumma, and Hamilton (1976) and extracellular polypeptides, (Fogg and Westlake, 1955). Glucose has been reported to reverse the toxicity of 3-(3,4-dichlorophenyl)-1,ldimethylurea (DCMU) possibly by meeting the organic carbon requirement, (Singh and Vaishampayan, 1978), although the present observation under laboratory conditions may not be directly extended to the

(ISSN: 1110-8649)

natural condition in which multi-parametric complex factors are operating. We report here the results of studies on the possible mode of action of 2, 4-dichiorophenoxyacetic acid (2, 4-D) on growth and heterocyst formation in nitrogen fixing blue-green alga *Anabaena* sp. and protection by glucose and tryptophan.

Materials and Methods

A laboratory experiment was conducted by using the filamentous N_2-fixing cyanobacterium *Anabaena* sp to study the effect of the most common used herbicide 2,4-Dichiorophenoxyacetic acid (2, 4-D) supplemented to th cyanobacterium culture medium with different concentration (0, 100, 500, 1000 and 1500 µg / mL) in the absence or presence of glucose and /or tryptophane (50 µg / mL) on the growth and heterocyst frequency. The tested organism was previously isolated by Ghazal (1991) from rice fields and was routinely propagated to axenic population in free nitrogen medium of Allen and Arnon (1955) at pH 7.5 by employing standard microbial techniques. The cultures were grown under fluorescent tubes light (approximately 3000 Lux on the surface) for 14 h a day at 28-32°C. Growth was estimated by following changes in absorbance of culture suspension at 660 nm in a spectrophotometer model 21 D (Bush and Lomb Co. USA). Specific growth rate k was calculated by the equation of Kratz and Myers (1955). Dry mass of 10- day old cultures was measured in 5-mL samples after oven drying at 80°C to a constant mass. Alga grown in an ammonium medium (1 mM NH_4C1) was used for heterocyst differentiation study (no heterocysts were formed here). Heterocysts were counted microscopically and their frequencies (averages of 10 random samples) expressed as the percentage of the number of vegetative cells after 4 d of incubation in N-free medium with or without chemicals (Vaishampayan, 1982). The chemicals used were of highest laboratory grade purity: 2, 4-D as sodium salt (Central Agricultural Pesticides Lab, Giza, Egypt), DL-tryptophan (BDH Chemicals) and glucose (BDH, England). Exponentially growing population was fragmented by glass beeds (aprox. 25 cells per filament). Liquid cultures containing 4 x 10^5 cells per mL of initial inoculum were grown in colorimetric flasks for direct colorimetric estimation of growth in triplicate. The observations are presented as X ± SD.

Results and Discussion

Effect on growth:

Results obtained in basal medium with or without added 2, 4-D showed that 2, 4-D stimulated the algal growth up to 100 µg mL^{-1}. A low inhibition was noted up to 500 µg mL^{-1} (Fig. 1). There was a consistent 2 to 4 d delay in the initiation of exponential growth in the presence of more than 100 µg mL^{-1} of the herbicide. Subsequent increase in herbicide concentrations inhibited algal growth

where 1500 µg mL^{-1} proved to be lethal as noticed by bleaching of the treated cultures within 4 days of incubation. The lethal concentration of 2,4-D was determined by transferring the treated cultures, grown for 10 days, after centrifuging and washing into fresh herbicide-free medium. Culture treated with 1500 µg mL^{-1} could not revive in fresh medium even after incubation for 20 days.

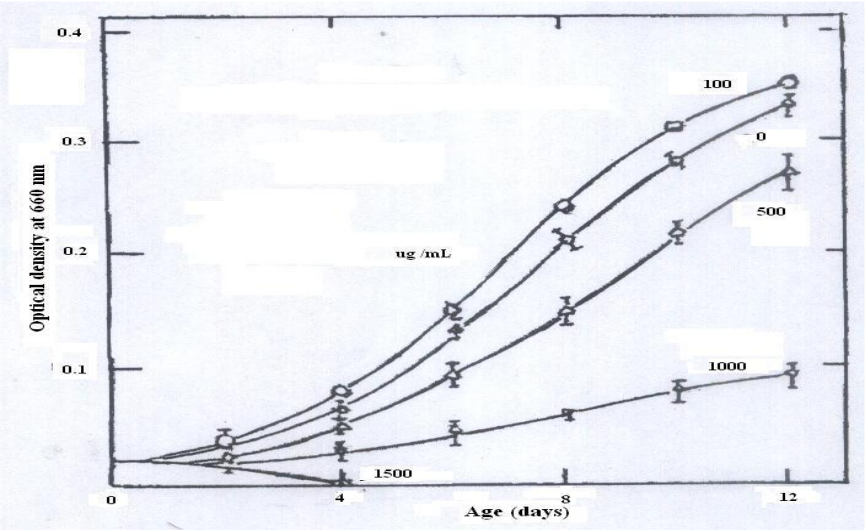

Figure (1): Growth response (absorbance A_{660}) of *Anabaena* sp. to different concentrations of 2, 4 – D (µg mL^{-1}, numbers at the curves)

A gradual decrease in the specific growth rate K was noticed at higher concentrations (Fig. 2). Dry mass of the herbicide-treated cultures also followed a similar trend as specific growth (Fig. 3). Stimulation in algal growth at lower doses (about 100 µg mL^{-1}) may due to the alternation in algal membrane permeability leading to an increase in the availability of nutrients or due to its action as growth hormone. The chlorinated phenoxy-acetic acid (2, 4-D) may act in plants as an analog of auxin, 3-indole-acetic acid, a natural growth promoting substance (Seiler 1978 and Pandher *et al*., 1994). It may be concluded that 2, 4-D at higher concentration adversely affects growth and cell multiplication, while lower concentrations seem to be stimulatory and/or non-effective for algal growth. These findings are in agreement with the results obtained by (Singh *et al*., 1988; Srinivasan *et al*., 1993 and Ismail *et al*., 1995). The concentrations of glucose and tryptophan which yielded optimum algal growth partially reduced the herbicide toxicity in the alga (Fig.2). Herbicide-induced lag phase in growth was removed

by the presence of these compounds in the medium. Thus glucose and tryptophan modify the herbicide toxicity even in the presence of lethal herbicide concentrations (Fig. 2).

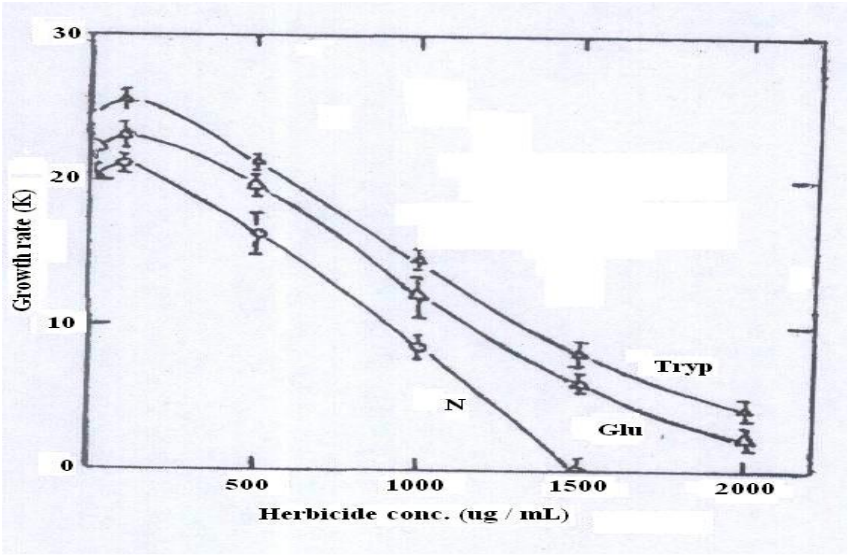

Figure (2): Effect of glucose and tryptophan on growth inhibition (specific growth rate K x 10^3) of *Anabaena* sp. By 2, 4 D (µg mL^{-1}); control N; glucose (500 µg mL^{-1}) and 50 µg mL^{-1}, Trp) supplemented media.

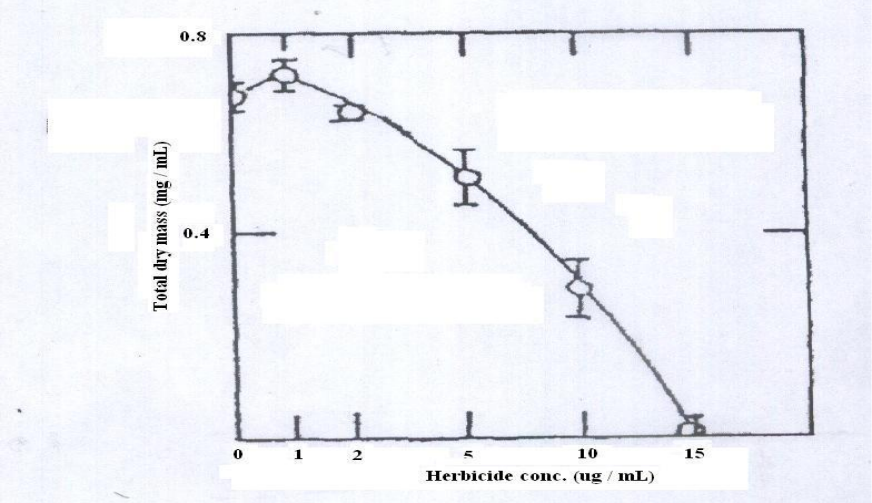

Figure (3): Total dry mass (mg/ mL) after 10 dayes of incubation of 2, 4 – D (µg/ mL x 10^2).

The observations suggest that the growth of the alga at higher concentrations of 2, 4-D is partially reversed by exogenous supplementation with glucose. The reversal could be attributed to glucose acting as an alternative source of reductant and/or carbon. The possibility that this substrate acts as a source of reducing power only would suggest assimilation of CO_2 even in the presence of 2, 4-D while the second alternative would mean a complete inhibition of photosystem II activity. The appearance of toxic symptoms in higher green plants treated with electron transport inhibitors can be prevented if the plants are supplied exogenously with oxidizable carbohydrates (Singh and Vaishampayan, 1978; Moreland, 1980 and Pandey and Tiwari, 1993). Thus 2, 4-D seems to affect electron transport in algal photosynthesis which circumvents the oxidizable carbohydrate glucose.

Heterocyst differentiation:

Ammonia-grown non-heterocystous filaments when incubated in N-medium for 4 days started to differentiate into heterocysts within 1 d and reached maximum frequency of 5.8% after 4 days. The growth-promoting concentration of the herbicide (100 µg mL^{-1}) stimulated heterocyst differentiation and the frequency reached 6.3% while at higher concentration (1000 µg mL^{-1}) the frequency was reduced to 2.0% (Table 1). Glucose considerably increased the heterocyst frequency (7.0%) without lag in the differentiation process and also showed commendable recovery in heterocyst frequency from 2.0 to 3.% at 1000 µg mL^{-1} besides reversing herbicide inhibition of growth and heterocyst formation at lethal concentration (Table 1). Tryptophan resulted in a two-fold increase in heterocyst frequency (12.0%) against control. Cultures grown in 2, 4-D (1000 µg mL^{-1}) - tryptophan. (50 µg mL^{-1}) complex medium did not produce mature heterocysts. The vegetative filaments were fragmented at the point of heterocyst development and detached (pigmented) heterocysts showed germination in situ.

Table (1): Effect of exogenous supplementation of glucose (500 µg mL^{-1}) and tryptophan (50 µg mL^{-1}) on the action of 2, 4-D- induced inhibition of heterocyst differentiation in *Anabaena* sp. (the average of 10 independent readings with their respective standard error (X ± SD).

2, 4-D µg mL^{-1}	Control	Glucose	Tryptophan
0	5.8 ± 0.14	7.0 ± 0.10	12.0 ±0.20
100	6.3 ± 0.20	8.4 ± 0.24 6.2 ± 0.11	14.4 ± 0.26
500	4.4 ± 0.43	3.4 ± 0.10	8.0 ± 0.14
1000	2.0 ± 13.0	3.4 ± 0.10	-b
1500	-a	3.1 ± 0.25	-b

Increase and/or decrease in N-growth of *Anabaena* with 2-4-D may be correlated with the frequency of formation of heterocysts, the specialized cells of nitrogen fixation under aerobic growth conditions. Thus, it is quite likely that growth and heterocyst differentiation was stimulated at lower concentration and diminished at higher concentration of 2, 4-D. Therefore, 2, 4-D can not be considered as an inhibitor of growth and heterocyst formation. Heterocysts serve as a good index for assuming that a chemical which inhibits heterocyst development under photoautotrophic condition may be an inhibitor of photosynthesis because as a photosynthetic inhibitor, it could cause changes in the normal-pattern of heterocyst development. The C: N ratio is known to control the pattern of heterocyst development in cyanobacteria (Fay, 1973). The fact that heterocyst development is sensitive to higher doses of 2, 4-D and readily reversed by exogenous supplementation with organic carbon, glucose, supported the view that toxicity may result directly from inhibition of photosynthetic carbon fixation as reported with DCMU, a known inhibitor of the light reaction and reductive assimilation of CO_2 in photosynthetically growing cultures of cyanobacteria (Pelroy *et al*., 1972).

Like glucose, tryptophan protects *Anabaena* from 2, 4-D toxicity by increasing growth yield and heterocyst formation. This may indicate that tryptophan is either incorporated into proteins or that triggers protein synthesis which initiates complete development of heterocysts (Mitchison and Wilcox, 1973). 2, 4-D negated this effect, supporting the idea that is involved in protein synthesis at higher doses, (Tiwari *et al*., 1982). Reduction in herbicide toxicity and inhibition of heterocyst development in 2,4-D - tryptophan complex medium might be due to formation of a 2,4-D- tryptophan complex (conjugate) which acts as auxin and /or nitrogen as reported with other amino acids in higher plants (Andreae and Good, 1957)

Mumma and Hamilton 1976; Pandey *et al*., 1984). Thus, the results suggest that heterocysts require a photo-synthetically fixed carbon supply and that glucose can effectively substitute for photosynthetically generated organic carbon for growth and heterocyst development as reported earlier with DCMU and propanil (3 ,4-dichloropropionanilide), (Vaishampayan et al., 1978; Pandey et al., 1984). Therefore, the action of 2, 4-D may be correlated with the mode of action of DCMU and propanil in relation to growth and heterocyst differentiation in *Anabaena* sp.

References

Allen, M. B. and Arnon, D. I. (1955). Studies of the nitrogen-fixing blue-green algae. I. Growth and nitrogen fixation by *Anabaena cylindrica* Lemm. *Plant Physiol*., **30: 366-371**.

Andreae, W. A. and Good, N. A. (1957). Studies in 3-indolacetic acid metabolism. *Plant Physiol*., **32:566-562**.

Daw, M. M. and Than, T. (1981). The effect of blue green-algae biofertilizer, *Azolla*, triple superphosphate and urea supplied individually and in combination on the yield of rice. In Proceeding of Agricultural Research Congress Rangoon, Burma (360 paper) 14-23 February.

El-Kholy, M. H. (1997). Effect of soil irrigation levels, algalization and nitrogen application on rice and soil properties. Ph.D. Thesis, Soil Since, Department of Soil, Faculty of Agricultural, El-Mansoura University.

El-Nawawy, A. S.; Ibrahim, A. N. and Aboul-FadI, M. (1968). Nitrogen fixation by *Calothrix* sp. as influenced by certain sodium salts and nitrogenous compounds. *Acta Agron. Acad. Sci. Hung*., **17: 323-327**.

Fay, P. (1973).The heterocysts, **p. 238** In: The Biology of Blue-Green Algae (N.G. Carr, B.A. Whitton, eds). Blackwell, Oxford.

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

Fogg, G. E. and Westlake, D. F. (1955). The importance of extracellular products of algae in fresh water. *Int. Assoc. Theor. Appl. Limnol*., **12: 219-225**.

Ghazal, F. M. (1991). Cyanobacteria strains isolated from rice in Nile- Delta. In: The Blue-green algae collection. Inter. Nat. Rice Res. Inst. (IRRI), Manila, Philippines, June, 1991. **p. 47**.

Ismail, B. S.; Siew, T. C. and Mushrifah, I. (1995). Effect of molinate and carbofliran on nitrogen fixation by *Azolla Pinnata*. *Microbes*., **62: 127-134**.

Kratz, W. A. and Myers, J. (1955). Nutrition and growth of several blue-green algae. *Am. J. Bot*., **42: 282-290**.

Mitchison, G. J. and Wilcox, M. (1973). Alteration in heterocyst pattern of *Anabaena* produced by 7-aza-tryptophan. *Nature New Biol*., **246:229-232**.

Moreland, D. E. (1980). Mechanisms of action of herbicides. *Ann. Rev. Plant Physiol*., **31: 597- 602**.

Mumma, R. O. and Hamilton, R. H. (1976). Amino acid conjugates in Bound and Conjugated Pesticide Residues (D.D. Kaufman, G.G. Still, G.D. Paulson, S.K. Bandal, eds). American Chemical Society, Washington (DC); ACS Symp. **Ser. 29: P 68**.

Pandey, A. K. (1985). Effects of propanil on growth and cell constituents of *Anabaena* calcicola. *Pesticide Biochem. Physiol*., **23: 157-162**.

Pandey, A. K. and Tiwari, D. N. (1993). Action of 2, 4-D on *Nostoc linckia*: Impact of glucose and tryptophan. *Folia Microbiol*., **31: 50-55**.

Pandey, A. K.; Srivastava, V. and Trwari D. N. (1984). Toxicity of herbicide Stam f-34 (propanil) on *Nostoc calcicola*. Z. Allg. *Mikrobiol.*, **24: 369-375**.

Pandher, M. S.; Gurmehar, K.; Gosal, S. K.; Gupta, R. P. and Kauer, G. (1984). Effect of herbicides on the growth and nitrogen fixation by blue-green algae. *Research and Development Reporter*.,**11: 9-13**.

Pelroy, R. A.; Rippka, R. and Stanier, R. Y. (1972).The metabolism of glucose by unicellular blue-green algae. *Arch. Mikrobiol*., **87:303-310**.

Seiler, J. P. (1978). The genetic toxicology of phenoxy acids other than 2, 4, 5-T. *Mut. Res*., **55: 197-206**.

Singh, A. L.; Singh, P. K. and Singh, P. L. (1988). Effects of different herbicides on the *Azolla* and blue-green algal biofertilizer of rice. *J. Agric. Sci*., **111: 177-183**.

Singh, H. N. and Vaishampayan, A. (1978). Biological effect of rice field herbicide machete on various strains of nitrogen fixing blue- green alga *Nostoc muscorum*. *Environ. Exp. Bot*., **18: 87-92**.

Singh, R. N. (1961). Role of Blue-Green Algae in Nitrogen Economy of Indian Agriculture. Indian Council of Agricultural Research, New Delhi.

Srinivasan, G.; Pothiraj, P. and Choudhry, G. K. (1993). Response of *Azolla* (*Azolla pinnata*) to rice (*Oriza sativa*) herbicides. *Ind. J. Agron*., **38:156-158**.

Stewart, W. D. P.; Hayastead, A. and Pearson, R. W. (1969). Nitrogenase activity in heterocyst of blue-green algae. *Nature*, **224: 227-230**.

Tel-Or, E. and Stewart, W. D. P. (1977). Photosynthetic components and activities of nitrogen fixing isolated heterocyst of *Anabaena cylindrica*. Proc. Roy.Soc. London, **Ser. B 193: P 61**.

Tiwari, D. N.; Pandey, A. K. and Mishra, A. K. (1981). Action of 2, 4-dichloro.phenoxyacetic acid and rifampicin on heterocyst differentiation in the blue-green alga, *Nostoc linckia*. *J. Biosci*., **3: 33-40**.

Tiwari, D. N.; Pandey, A. K.; Mishra, A. K. and Srivastava, V. (1982). Role of 2, 4-D on growth and macromolecular synthesis in a cyanobacterium, *Anabaena cylindrica*. *Ind. J. Bot*., **5: 111-116**.

Vaishampayan, A. (1982). Cu-Fe interaction in the N2 -fixing cyano bacterium Nostoc muscorum. *Microbios Letters*, **21: 17-23**.

Vaishampayan, A.; Singh, H. R. and Singh, U. N. (1982). Biological effect of rice-field herbicide Stam f-34 on various strains of nitrogen-fixing blue-green alga *Nostoc muscorum*. *Biochem. Physiol. Pflanzen*., **173: 410- 417**.

Watanabe, A. and Yamamoto, Y. (1971). Algal nitrogen-fixation in the tropics. *Plant and Soil*, **160: 403-413**.

Watanabe, I. and Liu, C. C. (1992). Improving nitrogen-fixing system and integrating them into sustainable rice farm. *Plant and Soil*, **141: 57-67**.

تأثير مبيد ال 2, 4 D على نمو السيابكتريا (*Anabaena* sp) فى وجود الجلوكوز والتريبتوفان

سكينة توفيق عبد الغنى * و السيدة على حسن**

**كلية العلوم- قسم النبات - جامعة القاهرة- الجيزة- مصر*

***قسم الميكروبيولوجيا الزراعية- معهد بحوث الأراضى والمياة والبيئة- مركز البحوث الزراعية- الجيزة- مصر*

فى تجربة معملية تم دراسة أثر مبيد الحشائش 2, 4 Dichlorophenoxy acetic acid على نمو السيانوبكتريا متمثلة فى طحلب الـ *Anabaena* sp وكذلك على تكون خلايا الهيتيروسيست وتكرارها وكذلك أثر تواجد أى من الجلوكوز والتريبتوفان على سمية المبيد التى تثبط نمو السيانوبكتريا. ويمكنتلخيص النتائج فيما يلى:

1- **شجع الـ 2,4 D نمو الطحلب حتى تركيز 100 ميكروجرام/مل بالمقارنة مع معاملة المقارنة.**
2- **كان هناك تأثير معاكس منخفض للـ 2,4 D عند تركيز 500 ميكروجرام/مل.**
3- **قل التأثير المثبط للمبيد على نمو السيانوبكتريا لمدة 2-4 أيام عند وجوده فى بيئة النمو بتركيز 500ميكروجرام/مل.**
4- **زيادة تركيز المبيد عن 500ميكروجرام/مل كان له تأثير مثبط حاد وصل الى أقصاه عند تواجده فى بيئة نمو السيانوبكتريا (*Anabaena* sp) بتركيز 1500ميكروجرام/مل.**
5- **شجع المبيد بتركيز 100ميكروجرام/مل تكون خلايا الهيتيروسيست وتكرارها بينما ادى وجود فى بيئة النمو بتركيز 1000ميكروجرام/مل الى اخفاض اعدادها وتكرارها.**
6- **أدى اضافة الجلوكوز الى زيادة تكرار خلايا الهيتيروسيست.**
7- **أدى اضافة التريبتوفان لبيئة نمو السيانوبكتريا الى زيادة تكرار خلايا الهيتيروسيست الى ضعف معاملة الكنترول.**
8- **أدى اضافة التريبتوفان بتركيز 50ميكروجرام/مل فى وجود المبيد بالبيئة بتركيز 1000ميكروجرام/مل الى عدم تكون خلايا الهيتيروسيست.**