Crude extracts of some fresh water Cyanobacteria have auxin-like activity on potato tissue culture

(Received: 03.02.2003; Accepted: 08.04.2003)

Sanaa M.M. Shanab*, Mahmoud M. Saker and Mahmoud H.M. Abdel-Rahman*****
* Botany Department, Faculty of Science-Cairo University,Giza-Egypt.
** Plant Cell and Tissue Culture Department, National Research Center,Giza-Egypt.
*** Botany Department, Faculty of Science, Fayum Branch, Cairo University.

ABSTRACT

The effect of crude extracts of ten fresh water cyanobacteria numbered (1-10) on the morphogenic responses of potato nodal explants in vitro was investigated. In order to study the auxin-like effect of cyanobacterial extracts, a new simple technique, employing potato tissue culture was applied. Both Murashige and Skoog (MS) basal medium or MS supplemented with 20% (v/v) algal crude extracts were used. MS medium containing 4 mg/l GA_3 was considered as positive control. The obtained data clearly indicated an auxin-like effect of crude extracts of five algal species (n° 1,4,5,7,10) out of the ten species used. A healthy strong potato plantlet (6.9-cm shoot length, 4-cm root length and 4 leaves per shoot) was proliferated from nodal explant (1 cm length) when cultured onto basal MS medium supplemented with 20% algal extract n°4. The growth parameters of plantlets onto positive control medium were 9 cm shoot length, 5 cm root length, and 7 leaves per shoot. Chromatographic analysis using TLC and GC/MS confirmed the presence of indolic substances in the five-algal extracts. It could be concluded that cyanobacteria have a strong auxin-like activity.

Abbreviations: IAA - Indole-3-acetic acid; IBA - Indole-3-butyric acid; ICA- Indole-3-carboxylic acid; IP - isopentenyl adenine; 2,4-D - 2,4-dichlorophenoxyacetic acid; GA3 - Gibberellic acid; GC/MS - Gas chromatography/Mass spectrometry; Kin - Kinetin; NAA - Naphthalene I-acetic acid; PAA- Phenyl acetic acid; TLC - Thin layer chromatography.

Key words: *Solanum tuberosum, micropropagation, algal extract, growth regulators, GC/MS*

INTRODUCTION

It is of interest to know whether or not the chemical substances acting as hormones in the most highly evolved land plants, such as the angiosperms, are already present in the more primitive plants such as algae. If such chemicals are present in algae, do they function as hormones, or do their hormonal function appear only in land plants? These questions have been answered to some extent concerning the angiosperm hormone IAA (Jacobs, 1986). Regarding the algal production of IAA, there have been numerous reports of auxins-like activity in different algal species. Du Buy and Olson (1937) recorded the presence of auxin in various parts of *Fucus vesiculosus*, and Van der Weij

(1937) reported the presence of growth substance in vesicles of *Valonia utricularis* and *V. macrophysa*, while Darsie (1939) demonstrated the presence of IAA in *Bryopsis muscosa*, and Van Overbeck (1940 a,b) found IAA in *Macrocystis* and other brown algae (*Fucus* and *Desmarestia*).

In this context, De Valera (1940) and Kylin (1942) reported that seawater taken from the zone of *Fucus* and *Ascophyllum sp*, stimulated growth of algae than seawater from other places or deeper water. Roborth and Thomas (1948) demonstrated the existance of growth substance in *Chlorella vulgaris* and its quantity increases when the alga was incubated in the dark. However Williams (1949) recorded the presence of a growth substance similar to IAA in the excised tissue of *Laminaria agardhii*. Bently (1958) was the first scientist who analyzed the culture medium of *Chlorella pyrenoidosa*, *Oscillatoria sp.* and *Anabaena cylindrica*, by extraction with organic solvents and separation by paper chromatography. He isolated an indolic substance which have a rate of flow near that of IAA and biologically active with *Avena* coleoptile test. Tandler (1962 a,b) reported the presence of indolic derivative in the thallus of different *Acetabularia sp.* before the formation of their Umbrella. Lefèvre *et al.* (1963) reported that fresh water cyanobacteria secreted in their synthetic medium substances which stimulated cellular multiplication.

Towards the identification of auxin-like substances using analytical methods, Tauts and Semenenko (1971), identified IAA in the extracellular metabolite of *Chlorella sp.* by its characteristic spectrophotometric absorption spectrum. Similarly, Abe et al. (1972, 1973, 1974) confirmed by MS the presence of IAA, ICA, PAA and P-hydroxy PAA in *Undaria pinnatifida*. They reported the presence of a substance with identical indolic properties (but they did not use Mass spectrometry for its identification) in *Ulva pertusa*, *Hizikia fusiforme*, *Gelidium amansii* and *Eisenia bicyclis*. Buggeln and Craigie (1971), Buggeln (1976) reported the presence of IAA in a marine algal species while, Fries and Aberg (1978) identified PAA in the extract of *Enteromorpha sp.* In 1982, Kingman and Moore reported the presence of IAA in *Ascophyllum nodosum* while, Jacobs *et al.* (1985) and Jacobs (1986, 1993) reported the presence of IAA and its catabolite dioxindole 3-acetic acid in the extract of *Caulerpa paspaloides*. This means that not only IAA is present and function as hormone in this green alga but also its catabolism follows the same pathway found in angiosperms (Reinecke and Bandurski 1987).

Recently, Hong *et al.* (1997) demonstrated that water extract of *Monostroma nitidum* enhanced cell growth of *Isochrysis galbana*, *Dunaliella salina* and inhibit the growth of *Enteromorpha prolifera* thalli. On the other hand, Cho *et al.* (1997, 1999) reported that methanol extracts of *Monostroma nitidum* inhibit growth of *Isochrysis galbana* and *Tetraselmis suecica*, while that of *Enteromorpha linzia* stimulates their growth.

This investigation was intended to search for gibberellins and or other endogenously produced growth regulating substances in some fresh water cyanobacteria which were isolated from Egyptian soils and water bodies.

MATERIALS AND METHODS

1- Algal cultures

The ten algal species used in this study (enumerated 1-10), and illustrated in Fig. (1) were previously isolated from soil and water samples of Ain Helwan, Egypt, in Spring 1999. Isolation and repeated subculturing on

BG11 & Bold's solid media leading to the separation of unialgal species, then purified by antibiotics (Stein, 1973) and identification according to Desikachary (1959); Bourrelly, (1970) and Prescott, (1978). Algal cultures were maintained on Bold's liquid nutritive medium and incubated at 20 °C under light-flux density of 10 μ (continuous illumination) for two weeks. The ten cyanobacterial species used in this study are:

(1)Nostoclinckia
(2)Nostocpaludosum
(3)Oscillatoriaacuminata
(4)Lyngbyavalderianum
(5)Anabaenaaffinis
(6) Nodularia implexa
(7) Plectonema nostocorum
(8) Anabaena iyengari
(9) Microcystis aeruginosa
(10) Schizothrix friesii

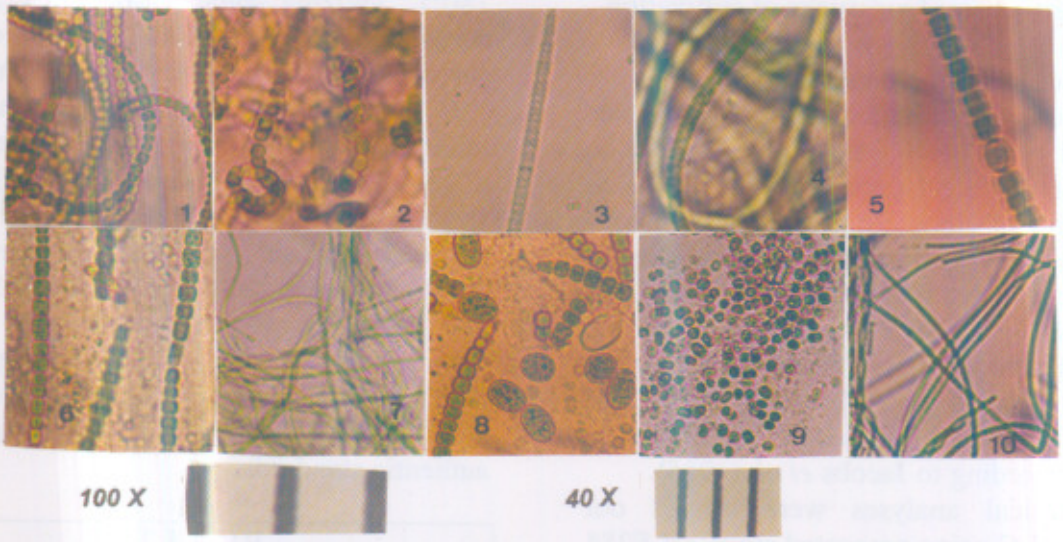

Fig. (1): IIIustration of the ten algal species numbered from 1-10 used in this investigation.

2- Preparation of crude algal extract

Algal crude extracts were prepared by the sonnication of 40 ml of algal cultures using 90k cycles for 10 min. (Fisher Sonic Dismembrator Model 150), then filtration through Watmann no.1 paper.

3- Potato Tissue Culture and Bioassay

To test the growth promoting activity in the ten algal crude extracts, potato tissue culture was chosen for bioassay. *In vitro* shoot cultures of potato (*Solanum tuberosum* L.) c.v. Sponta were initiated from meristematic shoot tip as described by Ducreux *et al.* (1986). To assess the effect of different algal crude extracts on morphogenic responses of *in vitro* potato nodal cuttings explants (1 cm length) were transferred to MS basal medium containin 20% of different crude algal extracts (1-10). MS basal medium

containing 20% of algal nutritive medium was used as -ve control. While MS medium containing 4 mg/l GA_3, which usually used for rapid mass micropropagation of potato in our lab.,was considered as +ve control and MS medium supplemented with GA_3 and algal nutritive components was used as control (n°11). Cultures were incubated in the growth chamber at 25 ± 2 °C, 16/8 light-dark photoperiod at light flux density of 25 µmol /m^2 /s and the duration of experiment was four weeks. Growth measurements including lenth of plants, n° of leaves and root length were recorded after four weeks of cultivation.

4- Physical and chemical analysis

Based on the results of bioassay, it was suggested that the growth regulators-like effect of crude algal extracts might be due to the presence of gibberellins-like and/or auxin-like substances. Accordingly, the procedure of extraction was intended primarily to look for Gibberellins in the cyanobacterial crude extracts. Extraction was carried out according to Abdel Wahab and Kobbia, (1976) and secondly to search for auxins in the same extracts according to Jacobs *et al.* (1985).

Chemical analyses were carried out firstly by TLC using precoated silica gel F254 by plotting 1 l of each algal extract and of 0.05 g/l of different authentic growth regulators (IAA, 2,4-D, NAA, IBA, Kin and GA_3). The following mobile phases were tested:

Hexane: Ethyl acetate: Acetic acid 16: 24: 1

Benzene: n-Butanol: Acetic acid 70: 25: 5 & 80: 15: 5

Confirmation of the observed results on the TLC and the identification of the separated active substance(s) were carried out using combined Gas Chromatography/Mass Spectrometery, at the Microanalytical center, Cairo University. Each sample was methylated with ethereal diazomethane (Vogel, 1975) and subjected to capillary GC using a Hewlett Packard 5890 series II Model MS 5988, containing 30mx 0.32 mm i.d. fused silica column with 100% dimethyl polysiloxane (Gµm) and using Helium as carrier gas. Five µl of samples were introduced onto the column via a cool-on-column injector. The GC temperature was increased from 125 to 280°C at a rate of 10°C/min. The column was coupled directly to the ion source of a Finnegam ion trap mass spectrometer. The source temperature was 180°C, ionizing voltage was 70 e.v. and trap current was 100 µA. The obtained chromatograms were searched for ions at m/z 130 (quinolinium ions) and m/z 101 (characteristic fragment of indole derivative of 3-substituted indole compound). Mass spectra of the chromatographic peaks containing those ions were compared with spectra stored in the computerized MS library and with published spectra of indoles (Ehmann *et al.*, 1975). Those having a close match to indolic compounds were compared with spectra obtained by GC/MS analysis of authentic standards.

RESULTS

Data presented in Table (1) summarize the growth measurements of potato nodal cutting, cultured for four weeks onto either MS medium supplemented with 20% different algal crude extracts, numbered 1-10 or controls (+ve and ve control). It was observed that there is no pronounced growth of the nodal cuttings cultured on MS medium containing algal extracts of *Nostoc paludosum* (2), *Oscillatoria acuminata* (3), *Anabaena iyengari* (8) and *Microcystis aeruginosa* (9), while algal extract of *Nodularia implexa* (6) permitted the proliferation of dwarf, shoot with only one node and leaf. Meanwhile,

crude extracts of algae no. 1, 4, 5, 7 and 10 (*Nostoc linnckia*, *Lyngbya valderianum*, *Anabaena affinis*, *Plectonema nostocorum* and *Schizothrix friesii*, respectively) have growth promoting effect more or less comparable to that of the positive control (MS + 4 mg/l GA3).

Table (1): Growth parameters of potato nodal cuttings cultured for 4 weeks on basal MS medium suplemented with 20% of different algal crude extracts.

Algal Number	Algal species	Shoot length (cm)	Root Length (cm)	Number of leaves	Description	Number of nodes
1	Nostoc linckia	6	3	5	Healthy, normal plantlet (comparable to + ve control)	5
2	Nostoc paludosum	-	-	-	----------	-
3	Oscillatoria acuminata	-	-	-	----------	-
4	Lyngbya valderianum	6.9	4	4	----------	4
5	Anabaena affinis	4.6	3	2	----------	3
6	Nodularia implexa	2	-	1	----------	1
7	Plectonema nostocorum	3.4	-	4	----------	4
8	Anabaena iyengari	-	-	-	----------	-
9	Microcystis aeruginosa	-	-	-	Lateral shoot premordium.	-
10	Schizothrix friesii	2.8	1	-	Etiolated plant	2
11	MS + GA3 + algal medium	7.8	2	4	Normal plantlet (as that of + ve control.)	5
+ ve cont.	MS + GA3	9	5	7	Strong, dark green plant with leaves.	7
- ve cont.	MS + algal medium	2	-	-	Etiolated plantlet	1

Each datum is the mean value of ten replicates.

The highest values of shoot and root length and number of leaves per plantlet (9, 5 and 7 cm, respectively) were recorded for nodal cuttings grown on positive control medium. However, the growth parameters of healthy plantlets proliferated from the nodal cuttings grown on MS medium supplemented with crude extracts of *Lyngbya valderianum* were 6.9 cm, 4 cm and 4, for shoot length, root length and number of leaves per plant, respectively. It was also observed that addition of only algal nutritive medium to basal MS medium (-ve control) led to the proliferation of rootless, etiolated, naked and dwarf shoots with one node (Fig. 2). Moreover, the addition of algal nutritive medium to GA3 containing medium (control no. 11) has no further promoting effect on growth, compared with positive control (Fig. 2).

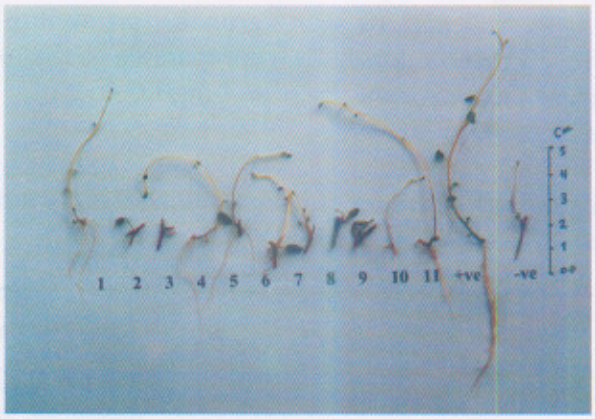

Fig. (2): IIIustration of the different morphogenetic responses of the Potato nodal explants cultured in vitro for weeks on:

1-10 MS + 20% of various algal crude extracts.
11 MS + 4 ml / GA3 + algal nutritive medium.
+ve control MS + 4 ml / GA3.
-ve control MS + algal nutritive medium.

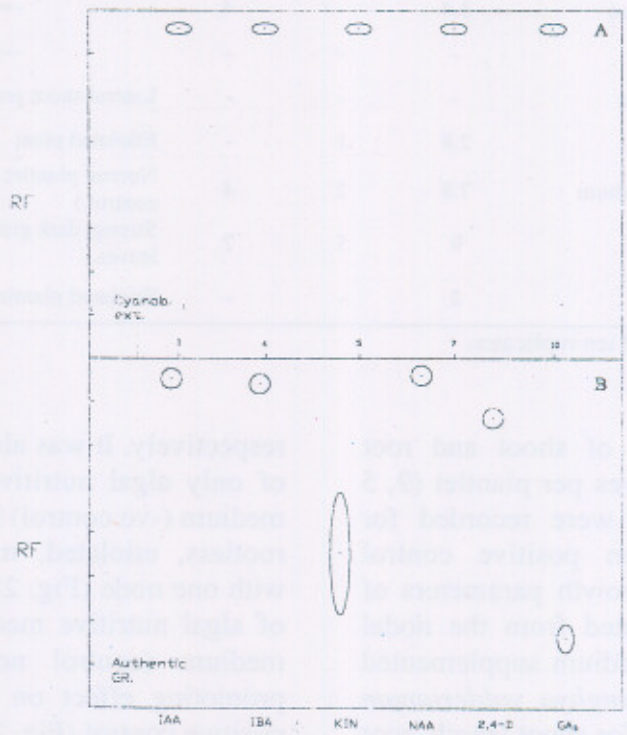

Fig. (3): Thin layer chromatography (TLC) of 1 µl of cyanobactrial extracts (A): 1- Nostoc linckia; 4- Lyngbya valderianum; 5- Anabaena affinis; 7- Plectonema nostocorum and 10- Schizothrix friesii with authentic growth regulators (B): IAA, IBA, KIN, NAA, 2,4-D and GA3 using precoated silica gel F254 and a solvent system of benzene: n-butanol: acetic acid (80:15:5).

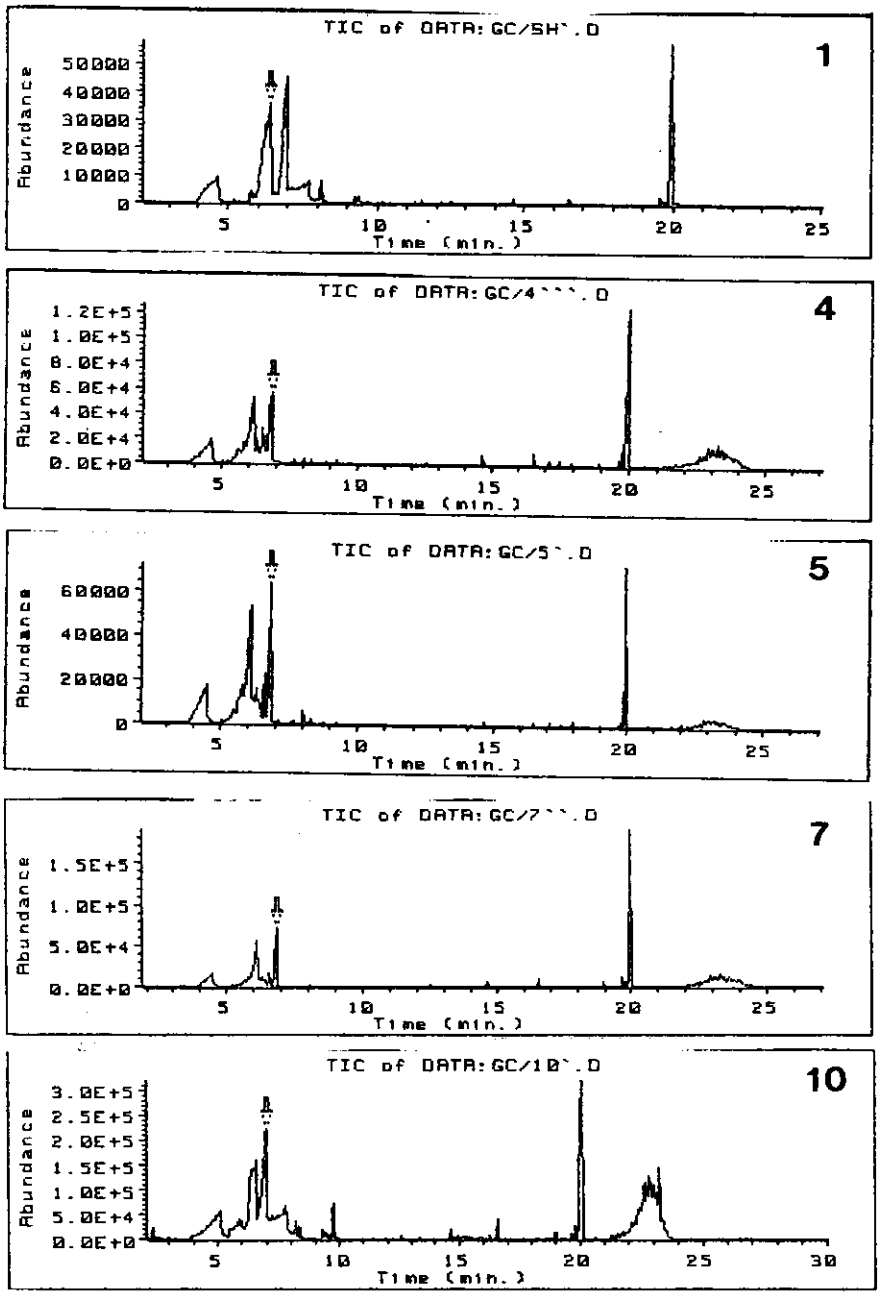

Fig. (4): The total ion chromatogram of methylated algal extracts: 1- Nostoc linckia; 4- Lyngybya valderianum; 5- Anabaena affinis; 7- Plectonema nostocorum and 10- Schizothrix friesii. The arrow (↓) point to the peak corresponding to the indolic substance in each chromatogram. Spectra were obtained on Hewlett Packard 5988 GC/MS.

It could be concluded from data summerized in Table (1) and presented in figure (2) that, crude extracts of some algae have growth promoting effects. The production of healthy, normal plantlets on MS medium supplemented with only crude extracts of some cyanobacteria, compared with those produced on either positive or negative controls gave an idea about the possible presence of endogenous growth substance(s) in the algal crude extract.

To confirm this observation on a chemical basis, the extracts of algae which gave positive effect on growth of potato nodal cultures, i.e. no. 1, 4, 5, 7 and 10 were analyzed using TLC. The separated spots of algal extracts on the TLC have comparable rate of flow to those of authentic indole compounds (not of Kinetin and Gibberellic acid) and react positively with the characteristic biochemical reagents of indoles (Fig.3). Confirmation of these results and identification of the endogenously produced indolic substance(s) by cyanobacteria were achieved by GC/MS. The total ion chromatograms of the five algal extracts show great similarity (Fig.4) and different indolic substances (IAA fragments) were separated from all algal extracts at comparable retention times (Table 2, Fig. 5,6 and 7).

Table (2): Retention times and molecular ions of the separated compounds from the methylated cyanobacterial crude extracts (number 1,4,5,7 and 10) using GC/MS.

Retention time (RT) mins	Sample numbers				
	1	4	5	7	10
4.471				102	
4.497			102		
4.607	102				
4.635		102			
6.117			131		
6.125				131	
6.216		131			
6.446	131				
6.534		145			
6.825			145		
6.833				145	
6.852		145			
6.981	145				
6.998					145
8.018			171		
8.148	171				
8.288		166			
9.272	163				
9.315					163
9.769					194

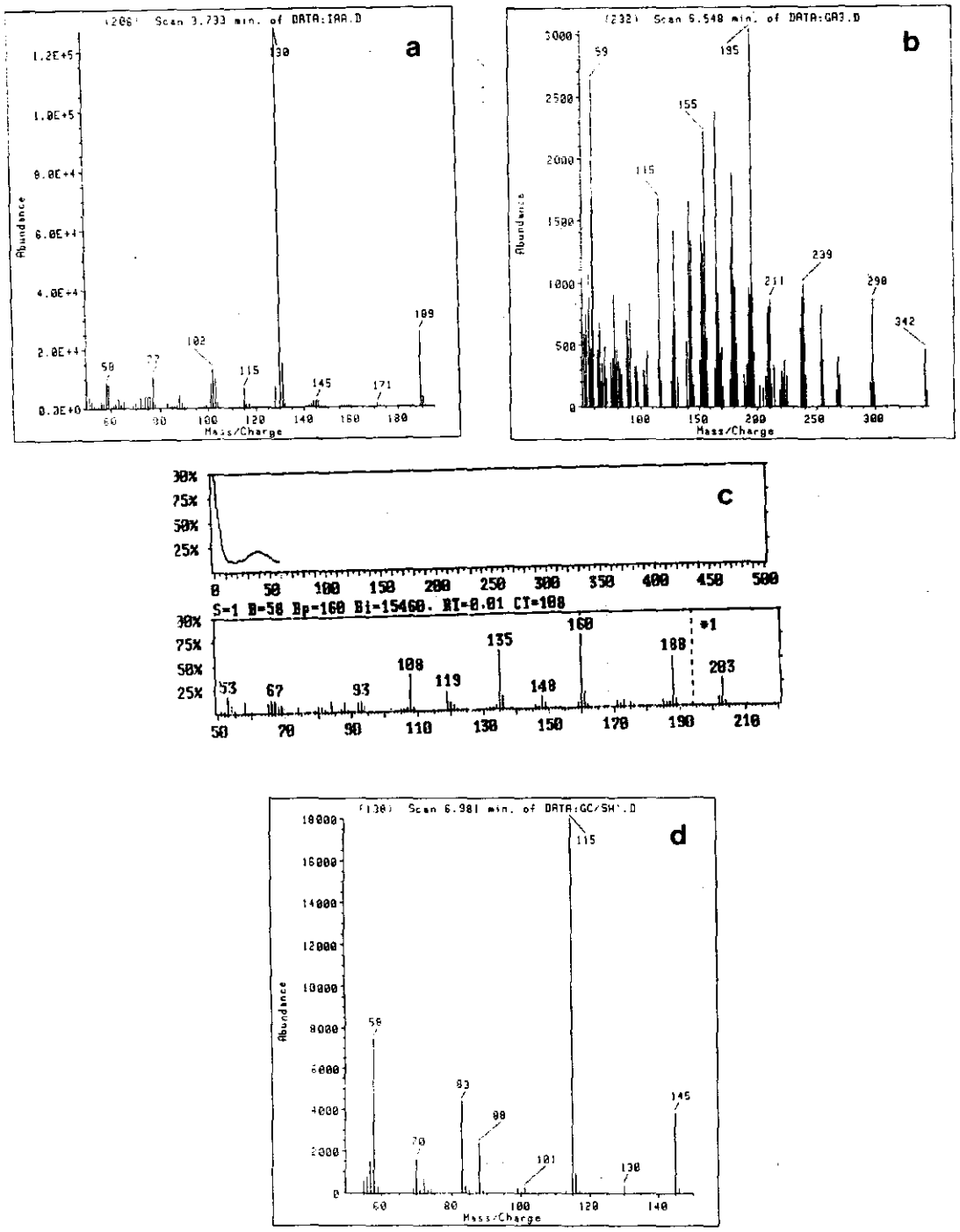

Fig. (5): Mass spectrum of methylated: a- authentic IAA; b- authentic GA3; c- authentic IP; d- fragment of indolic compound separated from the five algal extracts (1, 4, 5, 7, 10). Ion at m / z 145, 130, 115, and 101 correponding to molecular ion, quinolinium ion, basal peak and fragment characteristic of the 3-aubstituted indole compounds.

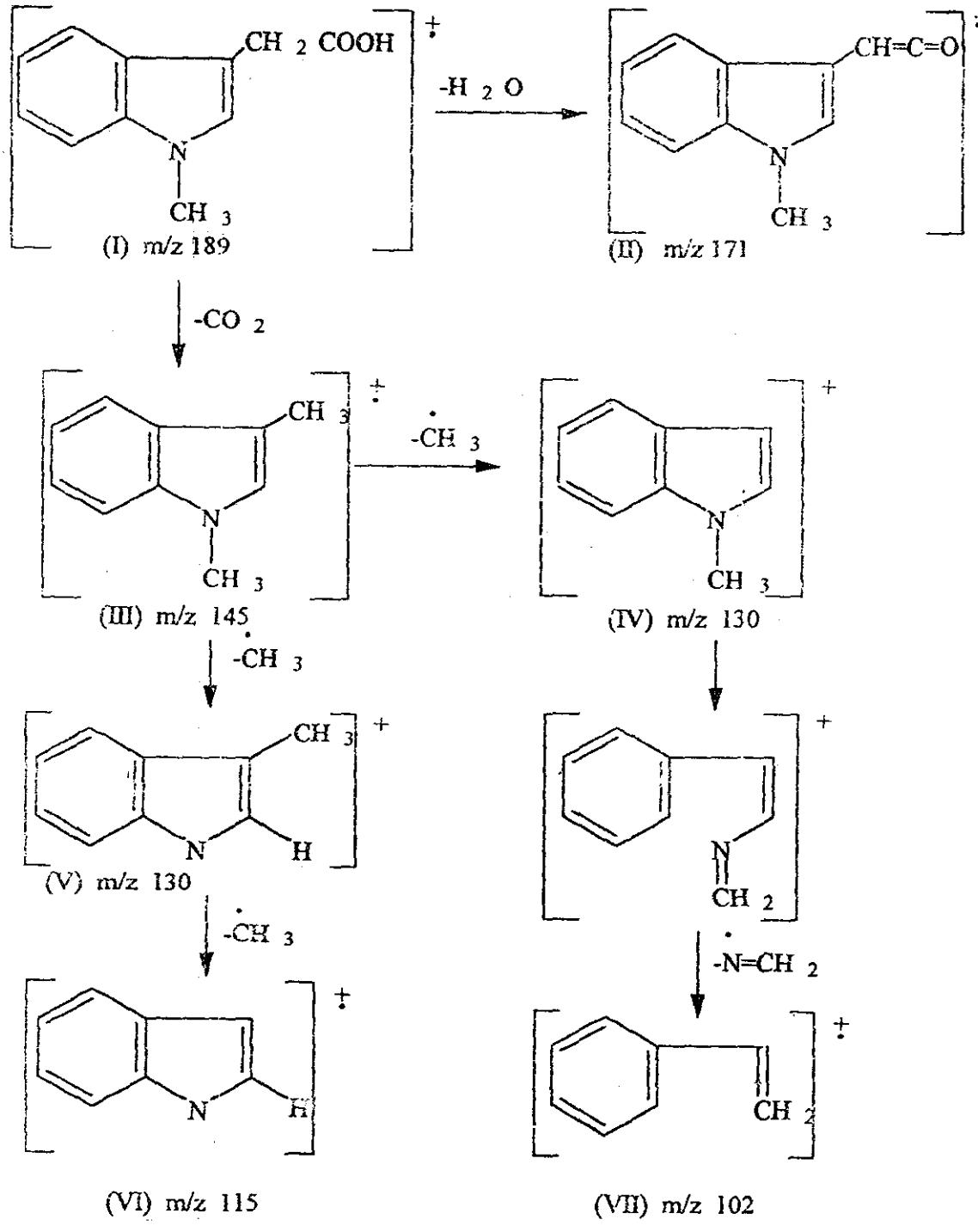

Fig. (6): IIIustration of the chemical structure of the methylated authentic IAA(I) and its different fragments (II, III, IV (=V), VI and VII) mostly produced GC/MS charts from the positively bioassayed cyanobacterial crude extracts (1, 4, 5, 7 and 10).

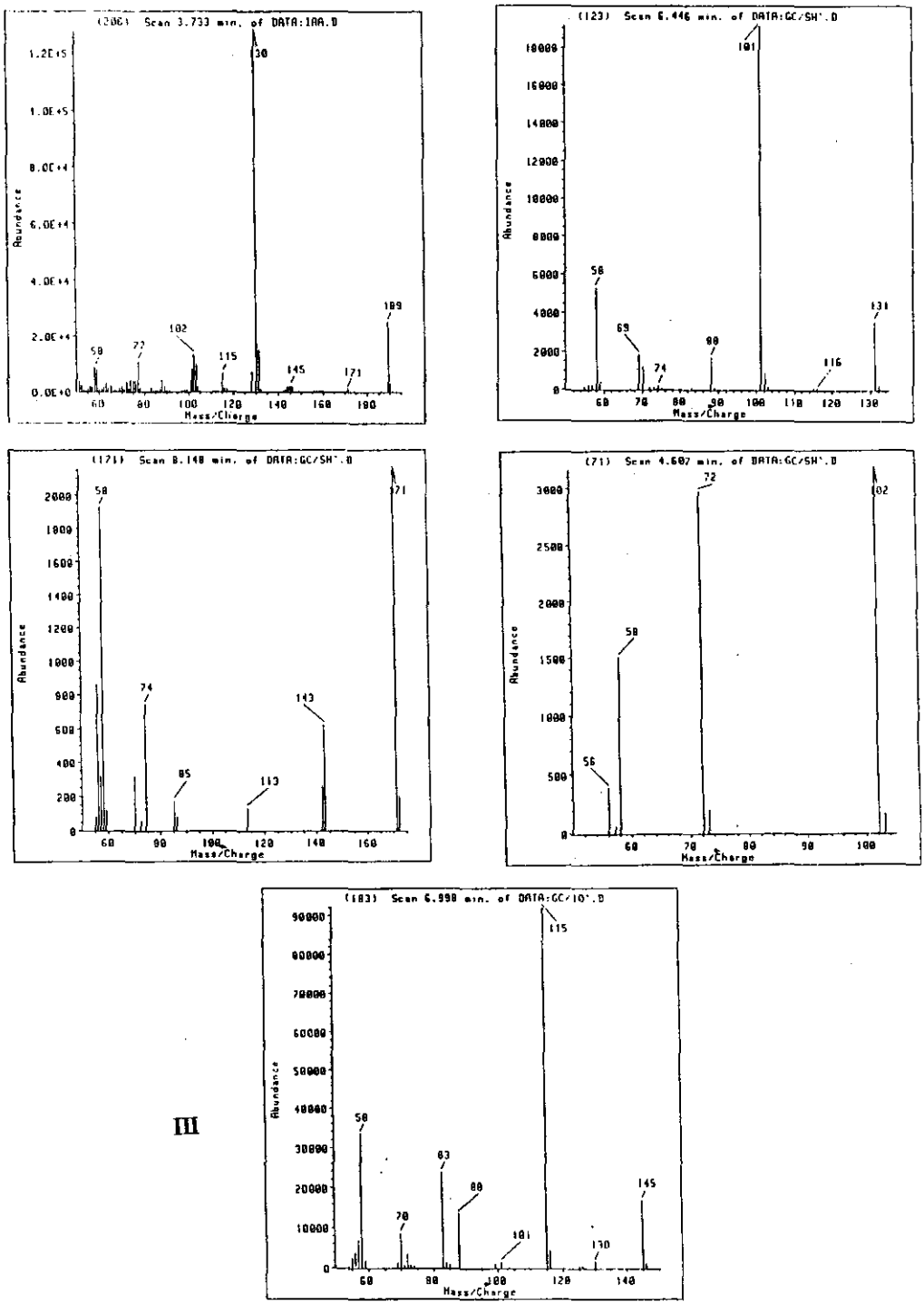

Fig. (7): Mass spectrum of the methylated authentic IAA (I) and its different fragments (II, III, IV(=V), VI and VII) produced in each of total ion chromatogram of five methylated cyanobacterial crude extacts [each fragment correspond to its chemical structure illustrated in Fig. (6)].

DISCUSSION

Auxins are important plant growth regulators which control various aspects of plant growth and development from the cellular to organ and whole plant levels. Despite the number of algal extracts showing auxin-like activities when tested in angiosperm bioassays, a few reports demonstrated that the responsible chemicals which act as endogenous hormones in higher plants, also actually function as growth substances in algae. Auxin-like activity was recorded mainly in numerous marine and fresh water algae, especially the brown and green species (Buggeln, 1976; Jacobs, 1993 and Cho *et al.*, 1999), while much fewer reports were concerned with cyanobacteria (Bently 1958, Lefèvre *et al.*, 1963).

Cyanobacteria were used worldwide for a long time in Agricultural practice in paddy fields (Watanbe, 1962 and El Nawawy 1972). The increase in crop yields as a result of algal inoculation can not only be attributed to the nitrogen-fixing property of cyanobacteria, but may be largely due to the growth regulating substances endogenously produced by these algae. This suggestion is greatly supported by the fact that non-nitrogen fixing species as *Phormidium sp.* and Oscillatoria sp. (Gupta and Shukla 1967, Gupta and Gupta 1970) stimulated the growth of rice. Moreover, it was found that cyanobacteria in addition to their stimulatory effect on plant growth, it enhanced the production of secondary metabolites of *Ambrosia maritima* (sesquiterpene lactones) and *Solanum elaeagnifolium* (glycoalkaloids). These mechanisms may be controled with or mediated by hormones (Saker *et al.*, 2000 and Shanab 2001). Results of the present study were in accordance with the previously published data that pointed to the presence of auxin-like substances in algae. Many scientists recorded the presence of different indole derivatives and its catabolites in different algal species, among them (Augier, 1976; Kingman and Moore, 1982; Jacobs *et al.*, 1985; Jacobs, 1986; Reinecke and Bandurski, 1987 and Jacobs, 1993).

Data of the present study also indicated that five cyanobacterial extracts have auxin-like activity which are confirmed by TLC (Fig. 3) and MS (Fig. 5) when bioassayed with potato nodal cuttings *in vitro* and not any other growth regulator as it was confirmed by comparing different authentic hormones with those of algal crude extract. Two species are nitrogen-fixers (*Nostoc linckia* (1) and *Anabaena affinis* (5), while the other three species are non-nitrogen fixers (*Lyngbya valderianum* (4), *Plectonema nostocorum* (7) and *Schizothrix friesii* (10)). The methylated algal extracts produced, not only comparable total ion chromatograms (Fig. 4 arrow ↓) but also the same indolic fragments at more or less the same retention times (Table 2) . All mass spectra of the five extracts exhibit ions at m/z 145, 130, 115 and 101, corresponding to molecular ions, quinolinium ions, basal peak and fragment characteristic of 3-substituted indole compounds, respectively (Fig. 5 d, Fig. 6 and 7 (III)). Other fragments of indolic nature were also separated from the five algal extracts of molecular ions at m/z 171, 131 and 102, as illustrated in Fig. 6, 7 (II, IV and VII).

This observation again confirms that the increase in crop yields as a result of algal inoculation can not only be attributed to the nitrogen-fixing property of cyanobacteria, but may be largely due to the growth regulating substances endogenously produced by these algae. It could also be concluded that the hormonal function of auxins appears not only in the structurally more complex land plants and the highly differentiated marine algae, but also in structurally simple Prokaryotic

cyanobacteria. Such algae can be used on a large scale to increase plant crop yields as in case of rice.

ACKNOWLEDGEMENTS

The authers are deeply grateful to Dr. Mohamed M. Youssef, lecturer of organic chemistry, Chemistry Department, Faculty of Science, Cairo University, for his valuable help in identifying the MS charts of different growth hormones and their characteristic fragmentation patterns.

REFERENCES

Abe, H., Uchiyama, M and Sato, R. (1972). Isolation and identification of native auxins in marine algae. Agr. Biol. Chem. 36:2259-2260.

Abe, H., Uchiyama, M. and Sato, R. (1973). Plant growth regulateos occuring in marine algae. 8th intern. Conf. Plant growth substances, Tokyo 26 aug-1 sep. abstracts.

Abe, H., Uchiyama, M. and Sato, R. (1974). Isolation of phenylacetic acid and its phydroxyderivative as auxin-like substances from *Undaria pinnatifida*. Agr. Biol. Chem. 38:897-898.

Abdel Wahab, A.M. and Kobbia, I.A. (1976). Production of Gibberellin-like substances by nitrogen-fixing blue-green algae. Bull.Fac. Sci., Assiut Univ., 5(3): 273-286.

Augier, H. (1976). Les Hormones des algues. Etat actuel des connaissances. 1-Recherche et tentatives D'identification des auxines. Bot. Mar. 19:127-143.

Bently, J.A. (1958). Role of plant hormones in algal metabolism and ecology. Nature 181:1499-1502.

Bourrelly, P. (1970). Les algues d'eau douce. & Cie 512 p.

Buggeln, R.G. (1976). Auxin, an endogenous regulator in algae? J. Phycol. 12:355-358.

Buggeln, R.G. and Craigie, J.S. (1971). Evaluation of evidence for the presence of Indole-3-acetic acid in marine algae. Planta 97:173-178.

Cho, J.Y., Lim, H.J., Jin, H.J., Whyte, J.N.C. and Hong, Y.K. (1997). Effects of seaweed extracts on cell growth of the feed algae *Isochrysis galbana* and *Tetraselmis suecica*. Proceedings presented at the 97 Joint Meeting of Korean Societies on Fisheries Sciences 225-226.

Cho, J.Y., Jin, H.J., Lim, H.J., Whyte, J.N.C. and Hong, Y.K. (1999). Growth activation of the Microalga isochrysis galbana by the aqueous extract of the seaweed Monostroma nitidum. J. appl. Phycol., 10: 561-567.

Desikachary, T.V. (1959). Cyanophyta. In: Indian Councile of Agriculture Research, Bombay, India.

De Valera, M. (1940). Note on the difference in growth of Enteromorpha species in various culture media. Kungel, Fysiografiska S. Ilskapels! Lund Frhandlingar 10(5): 52-58.

Du Buy, H.G. and Olson, R.A. (1937). The presence of growth regulators during the early development of Fucus. Amer. J. Bot. 24: 609-611.

Ducreux, G., Rossignol, L. and Rossignol, M. (1986). La Pomme de terre. In: La Recherche (Paris). 17(74): 193-203.

Ehmann, A., Bandurski, R.S., Harten, J., Young, N. and Sweeley, C.C. (1975). Mass spectrometry of indoles and trimethylsilyl-indole derivatives. Michigan State University, East Lansing, MI.

El Nawawy, A.S. (1972). Research program on nitrogen-fixing blue green algae in Agricultural Microbiology. Agr. Res. Rev. 50: 117-128.

Fries, L. and Aberg, S. (1978). Morphogenetic effects of phenylacetic and p-hydroxyphenylacetic acid on the green alga Enteromorpha compressa (L.) Grev. in axenic culture. Z. Pflanzenphysiol. 88:383-388.

Gupta, A.B. and Gupta, K.K. (1970). The effect of Phormedium fovealarum extract on growth and development of Pea seedlings. Labdev. J. Sci. Technol. Kanpur 8:151.

Gupta, A.B. and Shukla, A.C. (1967). Studies on the nature of algal growth substances and their influence on growth, yield and protein contents of Rice plants. Labdev. J. Sci. Technol. Kanpur 5: 162.

Hong, S.Y., Seo, G. M.; Jin, H. J.,Park, S.M. and Hong, Y.K. (1997). Effects on the tissue growth of the green alga Enteromorpha prolifera by seaweed extracts. Proceedings presented at the 97 Joint Meeting of Korean Societies on Fisheries Sciences 229-230.

Jacobs, W.P. (1986). Are angiosperm hormones present in, and used as hormones by algae? In Bopp, M. (ed.) Plant growth substances 1985, Springer-Verlag, New York, pp. 249-256.

Jacobs, W.P. (1993). A search for some angiosperm hormones and their metabolites in Caulerpa paspaloides (Chlorophyta). J. Phycol. 29, 595-600.

Jacobs, W.P., Falkenstein, K. and Hamilton, R.H. (1985). Nature and amount of auxin in Algae. IAA from extracts of Caulerpa paspaloides (Siphonales). Plant physiol. 78: 844-848.

Kingman, A.R. and Moore, J. (1982). Isolation, purification and quantitation of several growth regulating substances in Ascophyllum nodosum (Phaeophyta), Bot. Mar. 25: 149-153.

Kylin, H. (1942). ber den Einflu von glucose, Ascorbinsäure und Heteroauxin auf die Keimlinge von Ulva und Enteromorpha, Kungl, Fysiogr, Sällsk, i Lund Förhandl, 12: 135-148.

Lefèvre, M., Laporte, G. and Flandre, D. (1963). Sur la sécrétion par certaines cyanophycees de substances stimulant la multiplication cellulaire. C. R. Acad. Sc. Paris 256(1): 254-256.

Prescott, G.W. (1978). How to know the fresh water algae. Brown Company Publishers Dubuque, Iowa, U.S.A., pp. 12-267.

Reinecke, D.M. and Bandurski, R.S. (1987). Auxin biosynthesis and metabolism. In Daviers, P. J. (ed.) Plant hormones and their role in plant growth and development. M. Nijhoff, Boston, pp. 24-42.

Roborth, J.R. and Thomas, J.B. (1948). The synthesis of growth substances. I- The action of heteroauxin on the growth of Chlorella vulgaris and the influence of light on the production of growth substances. Konnitl. Ned. Akad. Wetenschap. Proc. 51:87-99.

Saker, M.; Shanab, S. and Khater, M. (2000). *In vitro* studies on *Ambrosia maritima.* I-Morphogenic responses and algal toxins elicitation. Arab J. Biotechn. 3 (2):217-224.

Shanab, S. (2001). Effect of fresh water cyanobacterial extracts on alkaloid production of the in vitro Solanum elaeagnifolium tissue culture. Arab J. Biotechn. 4 (1): 129-140.

Stein, J.R. (1973). Handbook of phycological methods culture methods and growth measurements. Cambridge University press. p62-63.

Tandler, C.J. (1962 a). A naturally occurring crystalline indolyl derivative in *Acetabularia.* Naturwissenschafter 40:213-214.

Tandler, C.J. (1962 b). Bound indoles in Acetabularia. Planta 59, 91, 407:91-107.

Tauts, M.I. and Semenenko, V.E. (1971). En Russe: Extraction et identification des substances physiologiquement actives de nature indolique dans les métabolites

extracellulaires de la Chlorella. Dokl. Akad. Nauk, S.S.S.R., 198(4): 970-973.

Van Overbeek, J. (1940 a). Auxin in marine algae. Plant Physiol. 15: 291-299.

Van Overbeek, J. (1940 b). Auxin in marine algae plants II. Bot. Gaz. 101: 940-947.

Vogel, A.J. (1975). A text book of practical Original Chemistry. 3rd ed. P969-971, English language book society and Longman group Ltd. London.

Watanbe, A. (1962). Effect of nitrogen fixing blue green algae Tolypothrix teuis on the nitrogenous fertility of Paddy soil and on the crop yield of Rice plants. J. Gen. Appl. Microbiol. 8: 85-91.

Williams, L.G. (1949). Growth regulating substances in Laminaria agardhii, Science110:169.

الملخص العربي

مستخلص بعض السيانوبكتيريا له تأثير شبه أكسيني على زراعة الأنسجة لنبات البطاطس

د.ثناء محمود متولي شنب*، د. محمود محمد صقر**، د. محمود حافظ عبدالرحمن***

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

**قسم زراعة الخلايا والأنسجة المركز القومي للبحوث الجيزة مصر.

***قسم النبات كلية العلوم جامعة القاهرة فرع الفيوم الفيوم.

باستخدام تقنية زراعة الخلايا والأنسجة و الوسط الغذائي موراشيج و سكوج (MS) ، ثم إضعافه ٢٠% من مستخلص عشرة أنواع من الطحالب الخضراء المزرقة (أخذت الأرقام ١-١٠) لدراسة النمو و التغير الشكلي لأجزاء عقديه مفصولة من نبات البطاطس و لقد تم اختيار هذا النبات لأنه من المعروف أن الهرمونات النباتية (حمض الجبريليك) يضاف للوسط الغذائي بغرض تحفيز تكوين النبات الكامل من الأجزاء العقدية. أضيف ٤ مجم/لتر حمض الجبريليك لبيئة MS مضاف إليه الوسط الغذائي للطحالب مكونا مرجع رقم (١١) بينما أضيف ٤ مجم/لتر حمض الجبريليك لبيئة MS فقط ليكون المرجع الموجب بينما بيئة MS مضاف إليه الوسط الغذائي للطحالب كونت المرجع السالب. و أوضحت النتائج التي تم الحصول عليها وجود تأثير هرموني لمستخلص خمسه أنواع طحلبية من العشرة التي تم استخدامها في هذه التجربة و ذلك بمقارنة النبات الصغير الناتج من نمو الأجزاء العقدية (طولها ١ سم) النامية على بيئة MS مضاف إليه مستخلص الطحالب الخضراء المزرقة بالنباتات المكونة على مرجع ١١ و المرجع الموجب و المرجع السالب. تم تحديد الهرمون النباتي الموجود بالمستخلصات الطحلبية باستخدام نوعين من الفصل الكروماتوجرافي ، GC/MS,TLC و التى اكرت وجود اكسينات خاصة اندول حمض الخليك و مشتقاته.