Acta Horti Bot. Bucurest. **33**: 35-39
Bucureşti 2006

THE INFLUENCE OF TWO BIOSTIMULATORS ON GROWTH IN UNICELLULAR GREEN ALGA *CHLORELLA VULGARIS*

LAZĂR Daniela Anca*, POLESCU Lucia* ,
VOICA Constantin*

Abstract: The dynamics of absorbance and number of cells of *Chlorella vulgaris* Beij./ml of nutritive medium treated with kinetin and indole-3-acetic acid were investigated for one culture cycle. The biostimulators were added in Arnon nutritive medium of algal suspension in increasing concentrations from 0.001μM to 1μM. Both in case of kinetin and of indole-3-acetic acid, it was observed a stimulation of the growth process for 1μM (in case of kinetin) and 0,1μM (in case of indole-3-acetic acid) concentrations between the 4th and the 6th days of the experiment. After a week of treatement, for all concentrations the obtained values were relatively close to those obtained for the control, both in case of kinetin and of indole-3-acetic acid. Linear relationships to transform the absorbance in cells number/ml were proposed for each biostimulator.
Key words: *Chlorella vulgaris*, kinetin, indole-3-acetic acid, growth, Romania.

Introduction

Beginning with the researches made by Kögl (1935) (quoted by Boldor et al. 1981) and C. G. Miller (1955) (quoted by Boldor et al. 1981) until the recent researches presented by Mauseth (1991), Salisbury and Ross (1992), Davies (1995), Raven (2003) it came out that the responses of higher plants to auxins may include: stimulation or inhibition of the organs growth depending on concentration, stimulation of the cell elongation and cell division, stimulation of flower parts growth etc. It was observed at higher plants, too, a stimulation effect induced by kinetin on cell division, on growth of lateral buds and on leaf expansion resulting from cell enlargement. Besides these processes, some other physiological processes are influenced both by auxin and by kinetin, especially at higher plants.

There are only few references about the influence of the biostimulators on some physiological processes of algae. Yin (1937) proved the capacity of auxin to increase the cells diameter of *Chlorella*. In the same study it was mentioned that after two weeks the initial auxin content from the culture medium is dropping with 50% and in three weeks the decrease may reach 80-90%. Besides this interesting information no data regarding dynamics of the *Chlorella* population was provided in that paper.

Recent studies pointed out a large variability of the cytokinin- and auxin-like activity in different species of *Cyanophyta* and *Chlorophyta* (Stirk et al. 2002). The variability of auxin metabolism in different algal groups and species was discussed by Cooke et al. (2002).

In comparative toxicity study of 40 herbicides on *Chlorella vulgaris* (Ma et al. 2002) it was found that the effect of the auxinic herbicides was among the lowest ones.

In this paper the effects of kinetin and indole-3-acetic acid on growth in unicellular green alga *Chlorella vulgaris* Beij. were investigated.

*Universitatea din Bucureşti, Facultatea de Biologie, Aleea Portocalelor nr. 1-3, 060101, Bucureşti, România
e-mail: daniela@botanic.unibuc.ro

Materials and methods

The installation for the cultivation of the unicellular algae needs favourable conditions of illumination, CO_2 supply and a suitable condition for mineral nutrition.

Chlorella vulgaris alga was cultivated on Arnon nutritive medium (Boldor et al. 1983).

The experiments were carried out in a chamber with artificial illumination of 8000 lx. In order to avoid mutual shadowing, the algal suspensions, in cylindrical glass recipients of 1000 ml, were permanent bubbled with steady stream of air produced by aquarium pumps. Thus, the algae were supplied with CO_2 for photosynthesis and were uniform illuminated because the agitation of the culture medium prevented their sedimentation. The cylindrical glass recipients were filled with the culture medium only 3/4 from their volume.

The culture medium was inoculated with an amount of algal biomass producing a 100000 cells/ml suspension in all experimental variants.

The ambient temperature varied between 21^0- 25^0C.

The initial culture of *Chlorella vulgaris* Beij. was provided by the Plant Physiology Laboratory of Institute of Biology from Bucharest.

The reactions of *Chlorella vulgaris* Beij. cells treated with kinetin and indole-3-acetic acid were investigated.

The biostimulators were introduced in the culture medium before inoculation and the concentrations of the tested biostimulator solutions varied from 0.001μM to 1μM.

The absorbance of the algae cultures were observed within the culture cycle with a "Cecil 1020" spectrophotometer at $\lambda = 676$ nm.

The number of algal cells was estimated with a Thoma haemocytometric mount.

Results and discussions

The influence of different concentrations of kinetin on growth of *Chlorella vulgaris* alga is presented in figure 1. For the treated variants a higher rate of growth than control was obtained in the first week of the experiment, excepting the 0,001μM concentration. The differences were not significant in the first three days of the culture cycle. For the concentration of 1μM kinetin a stimulating effect was noticed between the 4^{th} and the 6^{th} days of the experiment. The effects of 0,01μM and 0,1μM kinetin treatment on the cell number were relatively close to control in the same period of the culture cycle. After a week of treatement, for all concentrations the obtained values were relatively close to those obtained for the control.

The data for the dynamics of absorbance of *Chlorella vulgaris* suspensions (highly correlated with the number of cells per ml as shown bellow) under different concetrations of indole-3-acetic acid are presentented in figure 2. Also, for this case, a certain stimulation of growth was noticed between the 4^{th} and 6^{th} days of the experiment, but for 0,1μM concentration only, meanwhile the other concentrations remained close to control values.

Numerous cells countings (with the haemocitometer Thoma mount) were carried out in paralel with absorbance measurements. In both cases, liniar relationships with a significant (at p=0.05 level) R^2 were obtained. The R^2 was about 0.985 for the kinetin treatments (Fig. 3) and in the case of indole-3-acetic acid, the R^2 was about 0.831 (Fig. 4). So, it is possible a quick and precise estimation of the number of cells per ml based on absorbance.

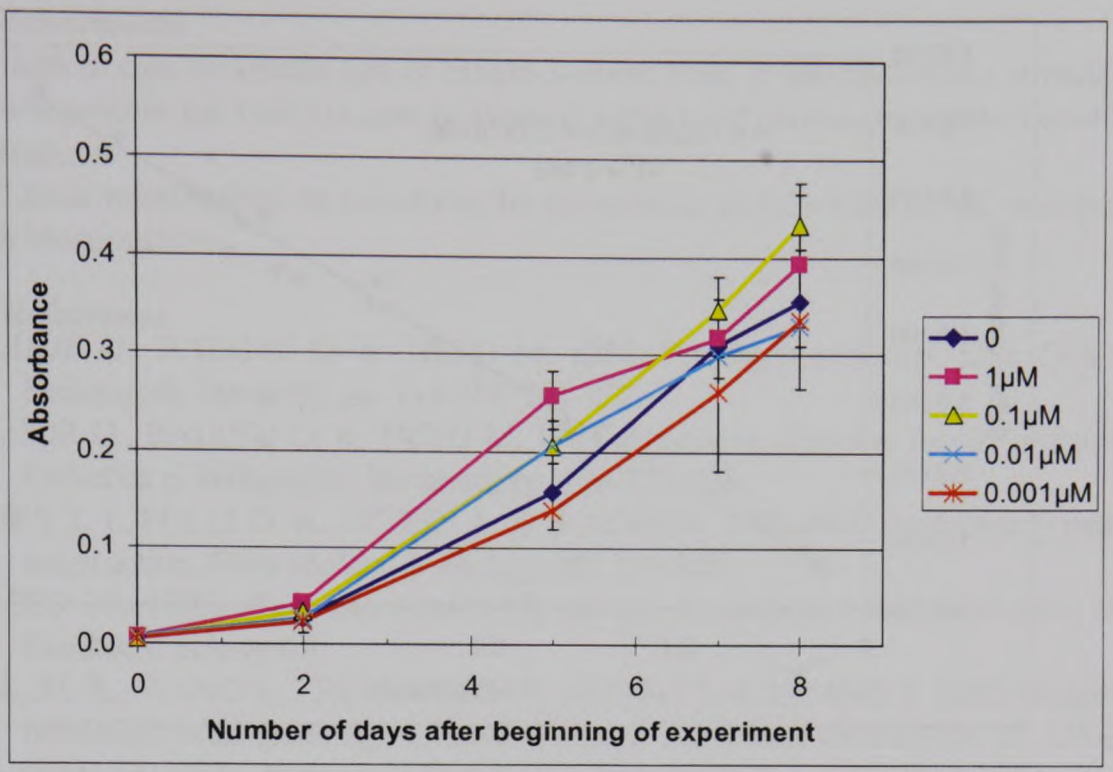

Fig. 1 Influence of kinetin on growth of *Chlorella vulgaris* (the vertical bars are representing two standard deviations)

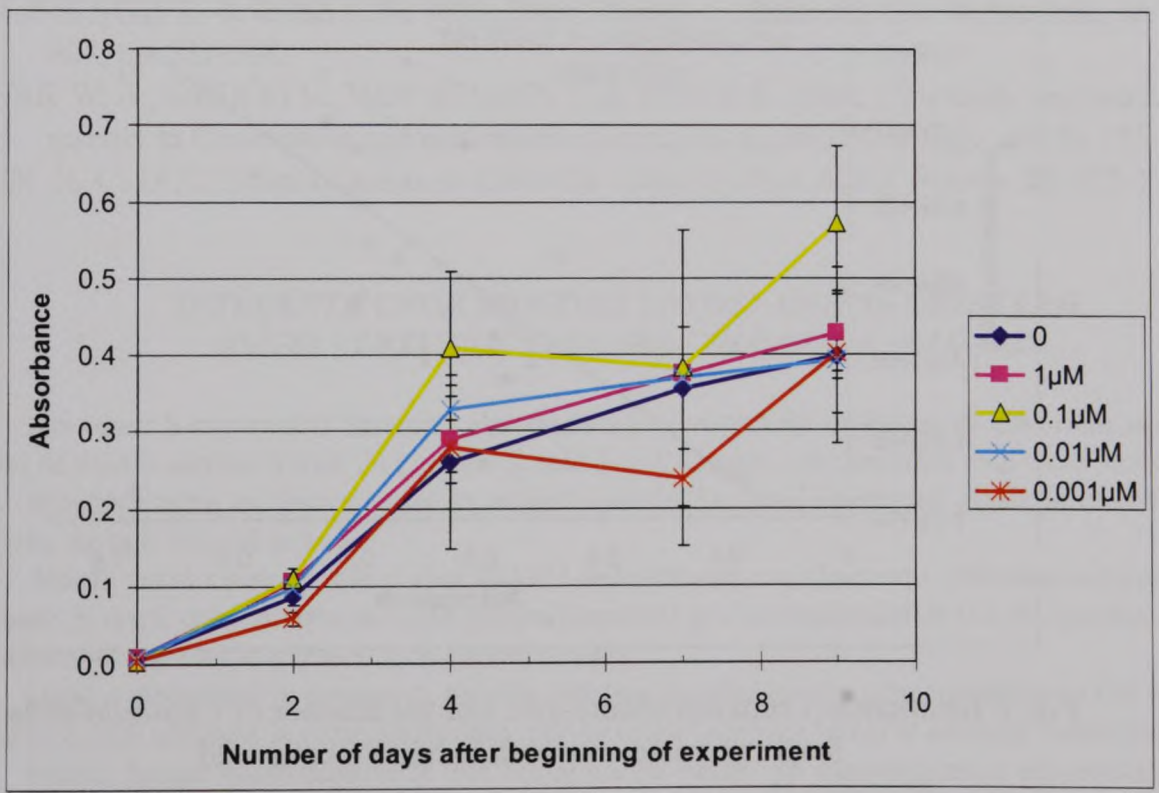

Fig. 2 The influence of the indole-3-acetic acid on growth of *Chlorella vulgaris* (the vertical bars are representing two standard deviations)

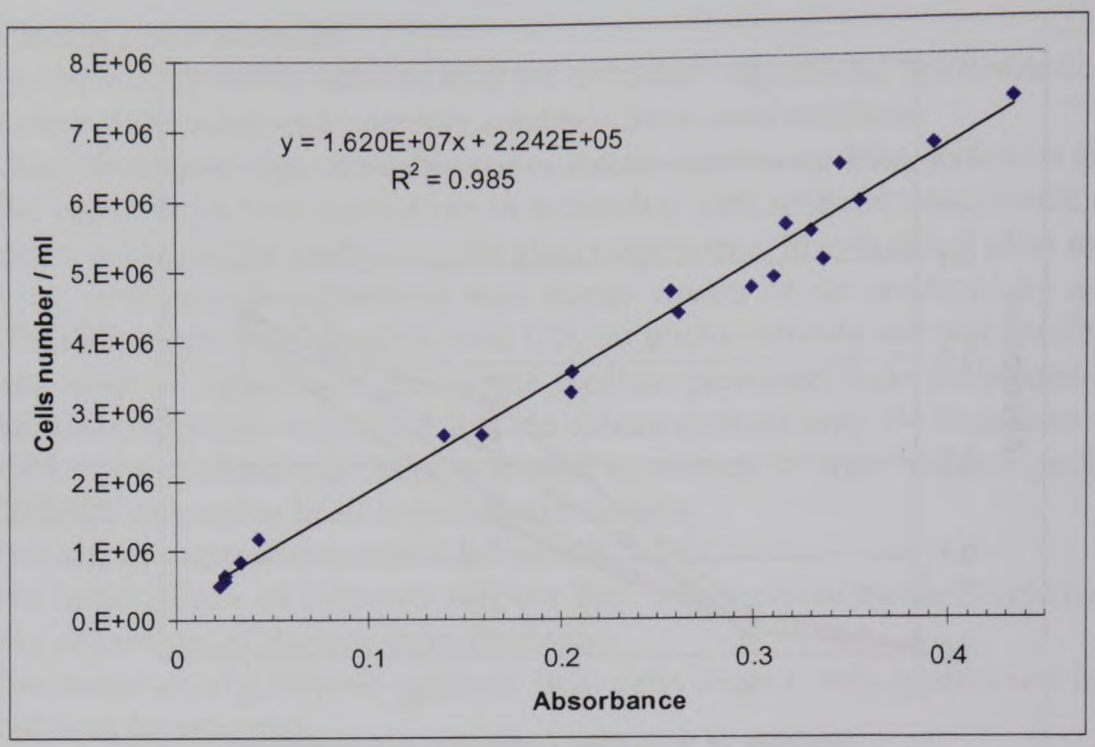

Fig. 3 Relationship between absorbance and the number of *Chlorella* cells/ml from the treatments with kinetin

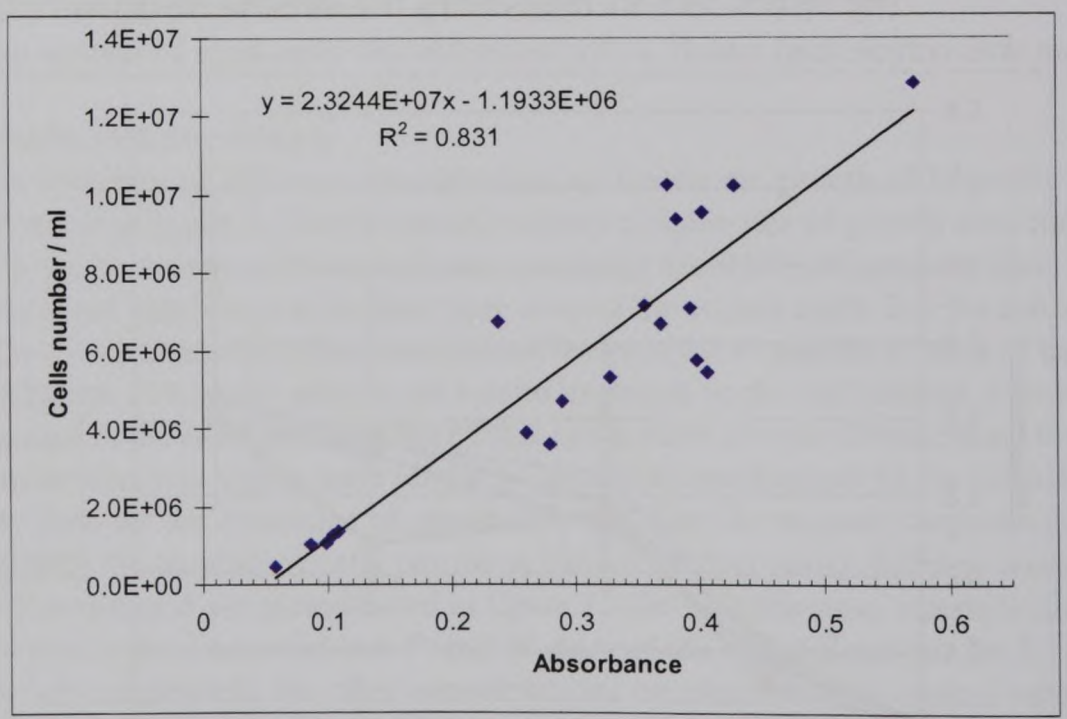

Fig. 4 Relationship between absorbance and the number of *Chlorella* cells/ml from the treatments with indole-3-acetic acid

Conclusions

Both in case of kinetin and of indole-3-acetic acid, it was observed a stimulation of the growth process for 1μM (in case of kinetin) and 0,1μM (in case of indole-3-acetic acid) concentrations.

Linear relationships to transform the absorbance in cells number/ml were proposed for each biostimulator.

References

1. BOLDOR O., RAIANU O. & TRIFU M. 1981. *Fiziologia plantelor*. Edit. Didactică şi Pedagogică, Bucureşti, pp. 212-218, 221-222.
2. BOLDOR O., RAIANU O. & TRIFU M. 1983. *Fiziologia plantelor, lucrări practice*. Edit. Didactică şi Pedagogică, Bucureşti, pp. 130-133, 138.
3. COOKE T. J., POLLI D. B., SZTEIN A. E. & COHEN, J. D. 2002. Evolutionary patterns in auxin action. *Plant Molecular Biology*, **49**: 319-338.
4. DAVIES P.J. 1995. *Plant Hormones: Physiology, Biochemistry and Molecular Biology*. Dordrecht: Kluwer.
5. MA J., XU L., WANG S., ZHENG R., JIN S., HUANG S. & HUANG Y. 2002. Toxicity of 40 herbicides to the green alga *Chlorella vulgaris*. *Ecotoxicol Environ Saf.* **51**: 128-32.
6. MAUSETH J.D. 1991. *Botany: An Introduction to Plant Biology*. Philadelphia: Saunders., pp. 348-415.
7. RAVEN P.H., EVERT R.F. & EICHHORN S.E. 2003. *Biology of Plants*. Sixth Edition, W. H. Freeman and Company Worth Publishers, pp. 675-701.
8. SALISBURY F.B. & ROSS C.W. 1992. *Plant Physiology*. Belmont, CA: Wadsworth., pp. 357-407; pp. 531-548.
9. STIRK W. A., ÖRDÖG V., VAN STADEN J. & JÄGER K. 2002. Cytokinin- and auxin-like activity in *Cyanophyta* and microalgae. *Journal of Applied Phycology*, **14** (3): 215 -221.
10. YIN H. C. 1937. Effect of auxin on *Chlorella vulgaris*. *Proc. N.A.S. Botany*, **23**: 173-176.

INFLUENŢA UNOR BIOSTIMULATORI ASUPRA CREŞTERII ALGEI VERZI UNICELULARE *CHLORELLA VULGARIS*

Rezumat: S-au urmărit dinamica absorbanţei şi a numărului de celule de *Chlorella vulgaris* Beij./ml de mediu nutritiv tratate cu kinetină şi acid 3-indolilacetic, pe parcursul unui ciclu de cultură.

Biostimulatorii au fost adăugaţi în mediul nutritiv Arnon al suspensiei algale în concentraţii crescânde, de la 0.001μM la 1μM.

Atât în cazul kinetinei, cât şi al acidului 3-indolilacetic, s-a observat o stimulare a procesului de creştere în cazul concentraţiei de 1μM (pentru kinetină) şi al concentraţiei de 0,1μM (pentru acidul 3-indolilacetic) între a 4-a şi a 6-a zi de experimentare.

După o săptămână de tratament, valorile obţinute în cazul tuturor concentraţiilor au fost relativ apropiate cu cele obţinute în cazul martorului, atât în cazul kinetinei, cât şi al acidului 3-indolilacetic.

Pentru fiecare biostimulator se pot folosi relaţii liniare de transformare a absorbanţelor în număr de celule/ml.

Cuvinte cheie: *Chlorella vulgaris*, kinetină, acid 3-indolilacetic, creştere, România.