THE EFFECT OF BENZENE AND ITS *CHLORELLA VULGARIS* METABOLITES ON THE GROWTH OF TOBACCO TISSUE

E. SZCZEPKOWSKA-KUŹNICKA, W. WARDAS*

University of Gdańsk, Department of Plant Physiology,
81-378 Gdynia, Poland

*Silesian Medical Academy, Institute of Chemistry and Physics,
Sosnowiec, Poland

(Received: February 2, 1995. Accepted: October 3, 1995)

ABSTRACT

The influence of benzene, phenol and catechol on the tobacco callus tissue (*Nicotiana tabacum* L. cv. Wisconsin No. 38) cultivated *in vitro* was studied. Although inhibition of callus tissue growth in relation to the control was observed in all variants, the benzene-induced inhibition was the most significant. When catechol and phenol were added to the cultivation medium together, callus tissue growth was similar to that of the control. The addition of catechol to the medium containing phenol presumably reduces the toxic effect of phenol on the callus tissue examined. Callus tissue growing in the presence of phenol, benzene and catechol became senescent more rapidly than in the control. Moreover microscopic examination revealed significant changes in the callus structure.

KEY WORDS: tissue culture, *Nicotiana tabacum*, benzene, phenol, catechol.

INTRODUCTION

It is evident that plant growth *in situ* is controlled by diverse chemical factors, among others by crude oil and its refinery products which are the most common pollutants of the sea. It is well known that a high concentration of oil pollutants significantly inhibits phytoplankton growth.

Unicellular algae have received particular attention. Winter et al. (1976) found that the addition of aqueous crude oil extracts to a microalgal culture medium inhibited the growth of blue-green algae and diatoms. The inhibitory effect of oil pollutants on the growth of phytoplankton was observed by Coffy et al. (1976), Kentzer et al. (1980), Kentzer and Tukaj (1984, 1985), Tukaj (1987).

Generally speaking, petroleum derivatives (including hydrocarbons) are known to have deleterious effects on microalgae growth (Wardas 1983). It has been found that benzene occurring in mineral oil was very highly toxic, even more so than other hydrocarbons (Wardas 1983, Wardas et al. 1987). Moreover, it has been shown that in the presence of *Chlorella* algae benzene undergoes a biotransformation, as a result of which, metabolites such as phenol and catechol are secreted into the medium by *Chlorella* cells (Wardas et al. unpubl). These metabolites demonstrated greater toxicity than benzene in relation to *Chlorella*. Furthermore, *in vitro* treatment of animal tissue fibroblasts with benzene, phenol and catechol retarded their growth intensity (Majnusz 1992, Wardas et al. 1992).

As regards higher plants, only a few papers on the influence of oil pollution on their growth have been published. In the author's own experiments (Kentzer, Szczepkowska 1988), the influence of aqueous oil extracts on the growth of ash (*Fraxinus excelsior*) embryos cultivated *in vitro* was investigated. The results indicated significant sensitivity of the embryos to oil pollution and showed considerable inhibition of both the shoots and roots of the seedlings in the presence of high concentrations of aqueous oil extract. Our investigations also suggested that these substances might be neutralized or degraded in the roots of seedlings.

Tobacco callus is known to be highly sensitive to growth modification factors and reacts in a specific manner towards them.

The primary aim of our work was to state whether benzene, as well as phenol and catechol as its metabolites, arising during its interaction with many plants, including *Chlorella* algae in water environment (Wardas et al. 1992), effect on tobacco callus tissue. Such a statement would be a base for further investigations about an application of callus tissue as a bioindicator of environment pollution caused by benzene and other hydrocarbons, present, among others, in crude oil and in oil-, and coal derivatives. In the case of phenol and catechol an application of callus tissue to their bioindication would mean a possibility of their detection also as environment pollutants independently of their arising in the process of benzene metabolism. We also intended to find out experimentally whether the concentrations of benzene, phenol

Fig. 1. Effect of benzene, phenol and catechol on the growth of tobacco callus (Lismaier and Skoog medium, 4 weeks, 25°C, $39 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$).

I – control medium, II – medium + benzene, III – medium + phenol, IV – medium + catechol, V – medium + catechol + phenol. Significant differences in relation to the control at $P = 0.01$.

and catechol, as they were in the presence of benzene metabolising *Chlorella* strain (Wardas et al. 1992), can be biologically active towards callus tissue. Tobacco callus is known to be highly sensitive to growth modification factors and reacts in a specific manner towards them.

MATERIAL AND METHODS

The experimental material comprised the pith callus tissue of *Nicotiana tabacum* L. cv. Wisconsin No. 38. This was grown on a sterile culture medium (Lismaier and Skoog 1965) for 4 weeks under continuous illumination at $39 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and a temperature of 25–27°C. After sterilization with a Sartorius filter (0.45 μm pores), solutions benzene, phenol and catechol were added to the culture medium in such quantities as to obtain the following final concentrations: benzene 0.5 mmol/dm³, phenol $24.5 \times 10^{-3} \mu\text{mol/dm}^3$ and catechol $5.4 \times 10^{-3} \mu\text{mol/dm}^3$. These concentrations were selected on the basis of a preliminary investigation in which 22.5 mmol/dm³ benzene had been added to the *Chlorella* cultivation medium. After 7 day of incubation the *Chlorella* medium contained the

remainder of the benzene (0.5 mmol/dm³) and its metabolites phenol ($24.5 \times 10^{-3} \mu\text{mol/dm}^3$) and catechol ($5.4 \times 10^{-3} \mu\text{mol/dm}^3$).

The experimental medium was prepared by adding 4 parts by volume of the callus tissue medium to one part by volume of the investigated solutions.

After 4 weeks of cultivation fresh and dry weights were measured. The results were evaluated statistically and least significant difference at $P = 0.01$ was calculated. Morphological and microscopic analyses of the tissue were carried out.

RESULTS AND DISCUSSION

The effects of benzene, phenol and catechol on the fresh and dry weight of callus tissue are shown in Fig. 1. Clearly, the applied quantities of these substances inhibited callus growth, most significantly in cultures treated with benzene. In this case, fresh weight increased only by 16.7% and dry weight by 24.9% in relation to the control. By comparison, the inhibitory effects of phenol and catechol were smaller. When phenol and catechol were added together, callus growth was

Fig. 2. Comparison of the effects of benzene, phenol and catechol on the growth of tobacco callus (Lismaier and Skoog medium, incubation time 4 weeks); 1 – control medium, 2 – medium + benzene, 3 – medium + phenol, 4 – medium + catechol + phenol, 5 – medium + catechol, R – root, S – shoot.

a

b

c

Fig. 3. Microphotograph of tobacco callus incubated for 4 weeks on a Lismaier and Skoog medium with
a – benzene,
b – phenol,
c – control. Mag. 800x.

almost the same as in the control. Addition of catechol to a medium containing phenol seems to diminish the toxic effect on tissue growth. The fresh weight increase in the medium containing catechol was similar to the variant where catechol was applied together with phenol.

It was found that in the presence of the examined substances callus tissue cultivation become senescent very rapidly. This effect was especially marked in the benzene variant and intensified as the culture proceeded. Likewise, Wardas et al. (1987) who investigated the effects of hexane, cyclohexane and benzene on growth algal, observed total retardation of culture growth and chlorosis at the beginning of the experiment. In the presence of benzene, chlorosis continued throughout the time of the experiment, whereas in the presence of hexane and cyclohexane, chlorosis ceased after 1-2 weeks of cultivation.

A significant influence of the investigated substances on callus tissue differentiation was observed (Fig. 2). Benzene and catechol caused changes in the callus tissue and influenced stem formation, but phenol stimulated rhizogenesis. Though surprising, these results are undoubtedly very interesting. The lipophilic properties of the investigated substances suggest that these may have provoked the changes in the cell membranes, which could have led to some modification of the membrane permeability of the examined callus and thus disturbed the hormonal balance observed in root and shoot formation.

Cytological examination showed that great changes in callus tissue structure had been caused by the investigated substances, especially by benzene and phenol. In the presence of benzene a significant increase in cell size in relation to the control and the formation of large tracheary elements were observed (Fig. 3a). In the presence of phenol the callus was friable and exhibited long, atypical cells (Fig. 3b). Similarly the presence of crude oil gave rise to abnormal cell enlargement in the unicellular alga *Scenedesmus quadricauda* (Tukaj et al. 1984). Culianez-Macia et al. (1987) found that the presence of p-dimethyl-aminobenzene (p-DMAB) in the medium accelerated and intensified ultrastructural changes in tobacco callus cells. Some of the changes in cell morphology caused by benzene and phenol observed in this study may also have been connected with some kind of transformation of cell structure.

Benzene, phenol and catechol influence callus causing specific morphological and anatomical changes. These effects were observed in the presence of studied substances in callus-tissue cultures at relatively low concentrations, the same as they occurred in the described previously work (Wardas et al 1992) concerning benzene metabolising by *Chlorella* algae. It means that the application of callus tissue can enable identification of just such, relatively low concentrations of benzene, phenol and catechol in the environment.

ACKNOWLEDGEMENTS

The authors thank Dr U. Majnusz (Silesian Medical Academy, Sosnowiec) for preparing the test solutions. We are grate-

ful to Prof. J. H. Rogozińska, Dr hab. D. Drozdowska (College of Agriculture, Bydgoszcz) and H. Mazur M. Sc. (University of Gdańsk, Gdynia) for helpful discussions and for reviewing manuscript.

LITERATURE CITED

- COFFEY J.C., WARD C.H., KING J.M., 1976. Effect of petroleum on the growth of freshwater algae. In: Proc. of the 31st Industrial Waste Conf., ed. by Arbor Science: 885-898.
- CULIANEZ-MACIA F.A., PRIMO-MILLO E., HERNANDEZ-YAGO J., GUERRI-SIERRA J., PRIMO-YUFERA E., 1987. Influence of p-dimethylaminobenzene and doxorubicin on tobacco cell growth (*Nicotiana tabacum* L.). Plant Growth Regul. 5: 155-164.
- KENTZER T., BURKIEWICZ K., SYNAK R., 1980. Growth responses of some marine algae to oil pollution. In: Proc. II Congr. of Feder. of European Societies of Plant Physiologists. ed. Univ. Santiago de Compostela: 430-431.
- KENTZER T., SZCZEPKOWSKA E., 1988. Growth responses of ash seedlings to oil pollution and gibberellin. Acta Phys. Plant. 10: 85-91.
- KENTZER T., TUKAJ Z., 1984. Some biological effects of oil pollution. Wiss. Zeitschr. Univ. Rostock 33: 31-32.
- KENTZER T., TUKAJ Z., 1985. Growth responses of *Scenedesmus quadricauda* to oil pollution at different temperatures and light intensities. Oceanologia 22: 41-49.
- LINSMAIER E.M., SKOOG F., 1965. Organic growth factor requirement of tobacco tissue cultures. Physiol. Plant. 18: 386-402.
- MAJNUSZ U., 1992. Aktywność biologiczna benzenu i jego metabolitów w hodowlach glonów *Chlorella vulgaris* oraz w hodowlach fibroblastów z wątroby myszy. Śląska Akademia Medyczna, Katowice. Ph. D. Thesis.
- TUKAJ Z., 1987. The effect of crude and fuel oils on the growth, chlorophyll "a" content and dry matter production of a green alga *Scenedesmus quadricauda* (Turp.) Bréb. Environmental Pollution 47: 9-24.
- TUKAJ Z., KENTZER T., BOHDANOWICZ J., 1984. The influence of fuel oil on the growth and cell morphology of *Scenedesmus quadricauda* (Turp.) Bréb. Proc. 14th Conf. Baltic Oceanographers, Gdynia 1984: 830-844.
- WARDAS W., 1983. Interakcja egzogennych węglowodorów z glonami *Chlorella* w środowisku wodnym. Politechnika Wrocławska, Monografie 22, Wrocław.
- WARDAS W., WARDAS M., MAZUREK U., WASZKIEWICZ E., MAJNUSZ U., 1987. Toksyczność wybranych węglowodorów względem glonów *Chlorella* w hodowlach laboratoryjnych. Roczn. PZH 38: 523-529.
- WARDAS W., WARDAS M., MAJNUSZ U., 1992. Biological Activity of Benzene and its Metabolites of *Chlorella vulgaris* Cultures in Fibroblasts Cultures from Mouse Liver. Polish Journal of Environmental Studies, 2: 27-30.
- WINTERS K., O'DONNELL R., BATTERTON J.C., VAN BAALEN C., 1976. Water-soluble components of four fuel oils. Chemical characterization and effects on growth of microalgae. Mar. Biol. 36: 269-279.

WPLYW BENZENU I JEGO METABOLITÓW OTRZYMANYCH
W HODOWLI *CHLORELLA VULGARIS* NA WZROST TKANKI KALUSOWEJ RDZENIA TYTONIU

STRESZCZENIE

Zbadano wpływ benzenu i jego metabolitów – fenolu oraz pirokatechiny (w stężeniach takich, w jakich wykryto je w środowisku hodowli glonów *Chlorella vulgaris* inkubowanych 7 dni w obecności benzenu) na wzrost, różnicowanie się oraz zmiany morfologiczno-anatomiczne tkanki rdzenia tytoniu hodowanej *in vitro*. Benzen obecny w podłożu wywoływał silną inhibicję wzrostu tkanki, natomiast fenol i pirokatechina znacznie słabiej hamowały ten proces. Łączne zastosowanie fenolu i pirokatechiny wykazywało słabszy efekt hamowania wzrostu tkanki aniżeli podane oddzielnie. Stwierdzono także istotny wpływ badanych związków na różnicowanie się kalusa. Obecność w podłożu benzenu lub pirokatechiny powodowała odróżnicowanie się tkanki w kierunku tworzenia części pędowych, natomiast fenol stymulował ryzogenezę. Obserwowano także zmiany w strukturze tkanki. Benzen stymulował powiększanie się rozmiarów komórek a fenol ich wydłużanie.

SŁOWA KLUCZOWE: bioindykacja, benzen, fenol, pirokatechina, tkanka kalusowa rdzenia tytoniu.