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BRIEF COMMUNICATION

Effect of 3-allyl-6-nitro-2-benzothiazolinone on algae and higher plants

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Abstract

In concentration range of 10^{-15} to 10^{-5} 3-allyl-6-nitro-2-benzothiazolinone (ANB) did not affect the alga *Chlorella vulgaris* L. and intact dicotyledonous plant *Vicia sativa* L. However, it stimulated growth and chlorophyll production in *Zea mays* L., showing different effects on individual plant organs, and in the callus obtained from the root of *Daucus carota* L. At high concentration (10^{-4} M), ANB inhibited all the characteristics studied.

Many chemical compounds of a high biological activity and selectivity (endogenous hormones as auxins, gibberellins, cytokinins, abscissins, but also synthetic growth regulators) participate in the regulation of physiological and morphological processes in plants. As these compounds may affect also the production of crop plants, new growth and metabolic regulators, for instance benzothiazolinone compounds (Gvozdjaková and Zemanová 1970, Sekerka and Sutoris 1985, 1986, Sutoris *et al.* 1986, Sidoóvá *et al.* 1990), are often studied. One of this compounds is 3-allyl-6-nitro-2-benzothiazolinone (ANB).

The aim of this work was to examine its effects on growth and chlorophyll (Chl) production in model plants (algae and higher plants) and to test its phytotoxicity.

The applied model plants were the alga *Chlorella vulgaris* L. (obtained from the Institute of Microbiology of Czechoslovak Academy of Sciences, Třeboň),and higher plants *Vicia sativa* L. (obtained from Slovosivo, Bratislava), maize (*Zea mays* L.) and a callus culture of *Daucus carota* L. (isolated from the carrot root in 1987).

The phytotoxicity of ANB was determined by measuring growth (a modified test according to Murín *et al.* 1980 and Sekerka and Sutoris (1985). Seeds of maize were imbibed for 24 h in 10^{-13}, 10^{-9}, 10^{-7}, 10^{-5} and 10^{-4} M ANB solutions and then the

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seeds germinated in vertical position in the dark at 25 ± 1 °C. After 72 h the length of roots and shoots was measured. For control distilled water was used. The fresh matter and Chl content in seedling shoots were determined after 6 d of cultivation in the hydroponic solution of Hoagland and Snyder (1966) at selected concentrations (10^{-8} and 10^{-6} M ANB).

C. vulgaris was stationary cultivated in the nutrition medium of Šetlík (1968) at 30 ± 1 °C, under irradiance of 6000 W m^{-2} with 16 h photoperiod and subcultivation time of 7 d. The effect of ANB on algae was determined by measuring growth rate (expressed as fresh matter of algal cells) and Chl content. Corresponding controls were cultivated in a pure nutrition medium.

The tissue culture of *D. carota* L. was cultivated on the liquid nutrition medium of Murashige and Skoog (1962) containing saccharose (2 %), kinetin (4.65 x 10^{-7} M), 2,4-dichlorophenoxyacetic acid (4.52 x 10^{-6} M), and 10^{-15} - 10^{-4} M ANB. The cultivation was caried out at 25 ± 1 °C, irradiance of 7 000 W m^{-2}, photoperiod of 16 h and subcultivation time of 21 d. The effect of ANB on growth index of the culture and Chl content was evaluated.

The Chl content was in all cases determined spectrophotometrically according to Inskeep and Bloom (1985) and calculated per dry matter unit. For extraction from algae and from maize seedling shoots *N,N*-dimethylformamide, from the tissue cultures 80 % acetone was used. The results were statistically evaluated using confidence interval for 95 % ($I.C._{0.05}$, Reisenaur 1970).

Table 1. Phytotoxicity test: Effect of 3-allyl-6-nitro-2-benzothiazolinone on growth in length of roots and stems of *Zea mays* L. after 72 h of incubation in the dark, expressed in % of the length of control plants. Means ± confidence intervals at $P = 0.05$.

Concentration [M]	Root [percent of control]	Stem
10^{-13}	160.23 ± 1.68	222.06 ± 12.26
10^{-9}	148.78 ± 1.34	220.20 ± 13.53
10^{-7}	128.23 ± 3.38	211.12 ± 10.85
10^{-5}	125.20 ± 4.98	194.24 ± 13.98
10^{-4}	52.67 + 3.19	92.15 + 5.63

The phytotoxicity test: Only the highest applied concentration of ANB (1 x 10^{-4} M) inhibited the growth of roots (Table 1). At low concentrations ANB stimulated the growth of roots (maximum at 10^{-13} M). Its effect on the growth of maize stem was similar (Table 1). The applied growth test for determination of toxicity (Murín *et al.* 1980) includes division of cells, their growth and differentiation. Similarly to cytokinins, auxins and gibberellins (Shtil'man *et al.* 1989) the toxicity of ANB with respect to plant growth was low.

The effect of ANB on the fresh matter and Chl content in maize was tested only at concentrations of 10^{-6} and 10^{-8} M 6 d after the ANB application a statistically significant stimulation of fresh matter (by 28 and 19%) and Chl content per dry matter unit (by 17 and 22%) was observed. Khadjieva *et al.* (1988) found a similar growth

The effect of ANB on green algae and plants: At concentrations of 10^{-13} to 10^{-5} M ANB did not statistically significantly affect growth and Chl content in cells of *C. vulgaris* L. At the concentration of 10^{-4} M a rather strong inhibitory effect took place, *i.e.* both the cell biomass and It is manifested with the decrease of cell biomass and chlorophyll content decreased approximately to one half. At concentrations of 10^{-13} to 10^{-5} M ANB did not affect the growth in length of roots and stems of vetch but again in the concentration of 10^{-4} M ANB exerted some inhibitory effect of (20% lower) on root growth and stem growth (10% lower).

Table 2. Relative effect of 3-allyl-6-nitro-2-benzothiazolinone, kinetin and 2,4-D and their combinations on properties of tissue culture of *Daucus carota* L. after 21 d of subcultivation, expressed as growth index and chlorophyll content. The values of reference growth medium (1st line) are taken for 100 %. Means ± confidence interval at $P = 0.05$.

Concentration [M]			Growth index	Chlorophyll
Kinetin	2,4-D	ANB	[% of control]	
4.6×10^{-7}	4.6×10^{-6}	--	100.00 ± 0.03	100.00 ± 3.99
4.6×10^{-7}		--	116.67 ± 1.96	111.89 ± 3.74
--	4.6×10^{-6}	--	101.96 ± 2.45	82.08 ± 3.74
--	--	1×10^{-13}	252.04 ± 0.03	386.02 ± 8.43
4.6×10^{-7}	4.6×10^{-6}	1×10^{-7}	82.04 ± 0.01	295.42 ± 4.22
--	--	1×10^{-4}	60.14 ± 0.03	72.98 ± 4.22
--	--	1×10^{-15}	137.47 ± 1.96	127.18 ± 3.75
--	--	1×10^{-13}	135.28 ± 3.43	125.39 ± 3.75
4.6×10^{-7}	--	1×10^{-7}	117.58 ± 1.96	122.71 ± 4.50
--	--	1×10^{-5}	111.45 ± 1.47	114.15 ± 3.75
--	--	1×10^{-4}	38.32 ± 1.16	--
--	--	1×10^{-15}	116.20 ± 2.94	116.95 ± 2.50
--	--	1×10^{-13}	131.37 ± 1.96	124.79 ± 3.75
--	--	1×10^{-7}	128.37 ± 2.94	95.53 ± 2.50
--	--	1×10^{-5}	110.29 ± 3.43	89.95 ± 1.25
--	--	1×10^{-4}	41.78 ± 4.11	--

stimulating effect (up to 148 % stimulation) with synthetic derivatives of urea (containing in their molecules 2 - 3 atoms of chlorine or one nitrogroup on the benzene ring) with respect to monocotyledonous plants in the concentration range 10^{-5} - 10^{-6} M, while dicotyledonous plants were not affected. Lazurkevich *et al.* (1983) measured the root and stem lengths of seedlings of mono- and dicotyledonous plants after application of 14-uracil and 6-azauracil analogues and their derivatives. Only one 6-azauracil compound group containing amino-group in its molecule increased the length of roots and stems of both mono- and dicotolydons by 17 - 37% and 15%, respectively.

Tissue culture of *Daucus carota* L.: The control culture was grown on the nutrition medium of Murashige and Skoog (1962) with kinetin, 2,4-D ANB and their combinations (Table 2). Excluding of 2,4-D from the control medium induced a moderate increase of both growth intensity (by 17%) and Chl content (by 12%).

Excluding of kinetin did not affect growth of the culture, but significantly decreased the Chl content (by 18%). 10^{-13} ANB in combination with kinetin and 2,4-D intensively stimulated growth and Chl content. Without 2,4-D the stimulating effect of ANB was significantly lower. ANB functioned like a stimulator also in systems without kinetin and 2,4-D. Optimum ANB concentration was always 10^{-13} M. According to Mohammad and Hassan (1988) chlorine derivatives of β-phenyl-3-indolylpropiophenone did not stimulate the growth of sunflower callus, howewer in the concentration of 10^{-7} M some of these compounds were able to replace 2,4-D, other ones kinetin.

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