THE INFLUENCE OF 4,6-BENZO[H]NAPHTHYRIDINE AND ITS METHYL DERIVATIVES ON THE ENZYMIC ACTIVITY AND CHEMICAL COMPOSITION OF CELERY (*APIUM GRAVEOLENS* L. VAR. *DULCE* PERS.) IN ITS GROWTH PHASES

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The influence of 4,6-benzo[h]naphthyridine and its derivatives 1-methyl- and 3-methyl-4,6-benzo[h]naphthyridines on the activity of peroxidase, catalase and acid phosphatase, as well as on the contents of chlorophyll, sugars (in general) and vitamin C in celery (*Apium graveolens* L. var. *dulce* Pers.) was investigated by pot experiments. Biologically active azaaromatics compounds, which were used in experiments, caused the changes of the enzymic activity and biochemical composition of celery leaves.

azaaromatic compounds; celery; peroxidase; catalase; acid phosphatase; chlorophyll; sugars; vitamin C

INTRODUCTION

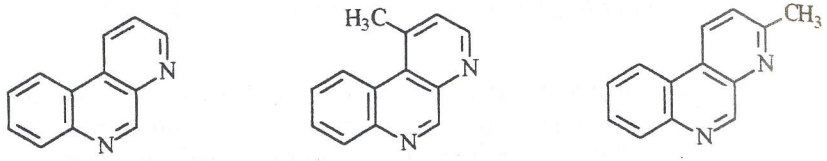
Agrotechnics are biologically active, they may change physiological and biochemical processes occurring in cultivated plants and disturb the regular enzymic activity in their growth phases (Cañal et al., 1988; Carroll et al., 1988; De Felipe et al., 1988). The topic of the research in this field are among others enzymes: peroxidase, catalase and acid phosphatase, having in view their important functions in the development processes of plants. The fundamental physiological role of catalase is the decomposition of hydrogen peroxide (Mac Rae, Ferguson, 1985); the removal of this compound is also the essential function of peroxidase (Upadhyaya et al., 1985). Acid phosphatase plays an important role in the production and transport of inorganic phosphates; these processes are a basis for a series of metabolic reaction (Duff et al., 1994).

Numerous investigations show that the changes of plant metabolism resulting from application of agrotechnics are to be seen also as the changes of the

biochemical composition of treated plants (Carroll et al., 1988; Salama, Awadalla, 1989; Schaller et al., 1991).

Having in view the above facts, it seemed of interest to study the influence of new chemical compounds (benzo[h]naphthyridines and its derivatives) on the enzymic activity and contents of various components responsible for the alimentary value of cultivated plants.

In the present work following azaaromatics, synthesised in the Department of Organic Chemistry of Pedagogical University of Częstochowa have been used: 4,6-benzo[h]naphthyridine (A), 1-methyl-4,6-benzo[h]naphthyridine (B) and 3-methyl-4,6-benzo[h]naphthyridine (C).



Employed benzo[h]naphthyridine and its derivatives are interesting from the biological point of view – exhibit a large antibacterial spectrum (Śliwa, 1978).

MATERIAL AND METHODS

In the years 1991 and 1992 the plant under investigation was celery (*Apium graveolens* L. var. *dulce* Pers.).

Experiments were carried out in the greenhouse. Plastic pots each of a surface of ca 0.1 m^2 were filled up with brown soil (7 l/pot) containing 1.7% of humus; the soil pH (KCl) being equal to 6.3. The contents of alimentary components before the beginning of cultivation was on average: 1.5 g N, 1.2 g K_2O, 0.8 g P_2O_5 and 0.6 g MgO/pot. During the whole vegetation period the ground moisture was kept on the equal level.

Celery was cultivated from seedlings grown on the seedbed and put into pots during the phase of 3–4 leaves in the second half of May.

The investigated compounds A–C were applied at two concentrations: 0.01% and 0.1%; the spray with these species was made two weeks after seedling of celery in pots. The amount of the considered compound A–C was ca. 20 cm^3 of the solution of a given concentration per pot (i.e. 0.02 and 0.2 g of A–C/1 m^2 surface).

The plant material for experiments was taken three times in periods of one month, beginning from July 1. For analysis three samples of each set composed of twenty fully grown up leaves were taken. The enzymic activity along with the content of chlorophyll and of vitamin C have been determined in the fresh plant material, the contents of sugars in the material kept in the dryer at 95–100 °C for three hours.

The activity of peroxidase [EC 1.11.1.7] was determined with o-dianisidine as H-donor (Gardiner, Cleland, 1974). The relative, elementary enzymic activity is an absorbancy increase per minute (ΔA/min). The activity of catalase [EC 1.11.1.6] was determined by measurement of the amount of decomposed hydrogen peroxide in mg/1 g of the fresh mass of leaves during 1 minute, at 30 °C, according to Bergmeyer (1963) methods. The activity of acid phosphatase [EC 3.1.3.2] was found by determination of phosphorus generated from sodium β-glycerophosphate by enzyme contained in 1 g of fresh mass of plant leaves, according to methods described by Brandenberger and Hanson (1953).

Chlorophyll a and b as well as the total chlorophyll (a+b) were determined by spectrophotometric method (Bruinsma, 1963), vitamin C by Tillmans method involving the reducing properties of L-ascorbic acid, and the content of sugars in general by Luffa-Schoorla method (given by Rutkowska, 1981).

The results were worked out statistically using variance analysis (the Fisher-Snedecor test *F*) and the Tukey test.

RESULTS

The influence of 4,6-benzo[h]naphthyridine (A) and its derivatives: 1-methyl- and 3-methyl-benzo[h]naphthyridines (B, C) on the activity of peroxidase, catalase and acid phosphatase in three growth phases of celery is given in Table I. Azaaromatic compounds, which were used in experiments, caused the changes of the enzymic activity of celery leaves. Here the changes are dependent so on the kind of compound used, as well as on its concentration and growth phase.

With the physiological age of celery, the increase of enzymic activity of peroxidase took place. In all growth phases of plant a stimulation influence on the peroxidase contents, independent on the kind of used azaaromatic, was observed. As compared with the control plants, the increase of the enzymic activity of peroxidase (average of action of A–C, average of growth phases) was 39% at 0.01% concentration and 35% at 0.1% concentration.

Along with the increase of the peroxidase activity, with the physiological age of plants, the decrease of the catalase activity was observed. In all plants treated with azaaromatic compounds during the whole vegetation period the rice of the catalase activity as compared with that in the control plant took place. The increase of the catalase activity (average of action of A–C and

I. The influence of 4,6-benzo[h]naphthyridine (A) and its methyl derivatives (B, C) on the activities of peroxidase, catalase and acid phosphatase in celery leaves in different growth phases

Compounds and their contents	Peroxidase (ΔA/min) growth phases				Catalase (mg H_2O_2/g min) growth phases				Acid phosphatase (mg P/g) growth phases			
	I	II	III	mean	I	II	III	mean	I	II	III	mean
Control	0.062	0.066	0.078	0.069	6.28	7.29	3.83	5.80	1.729	2.979	3.709	2.806
A – 0.01%	0.090	0.112	0.081	0.094	7.29	7.79	4.12	6.40	1.750	3.646	3.854	3.083
B – 0.01%	0.100	0.105	0.097	0.100	7.04	8.04	6.18	7.09	2.135	3.136	4.062	3.111
C – 0.01%	0.072	0.095	0.114	0.094	8.29	7.79	6.18	7.42	2.625	4.448	3.719	3.597
A – 0.1%	0.101	0.108	0.100	0.103	8.55	8.04	5.88	7.49	2.334	3.709	3.844	3.295
B – 0.1%	0.083	0.088	0.084	0.085	9.53	8.29	5.00	7.61	2.104	4.240	4.052	3.465
C – 0.1%	0.075	0.090	0.110	0.092	7.79	8.55	6.18	7.51	1.854	3.823	3.584	3.087
$LSD_{0.05}$	for compounds – 0.005				for compounds – 0.61				for compounds – 0.042			
$LSD_{0.05}$	for phases – 0.008				for phases – 0.94				for phases – 0.064			

average of growth phases) was 20% at 0.01% concentration and 30% at 0.1% concentration.

The analysis of changes of acid phosphatase activity in plants treated with 4,6-benzo[h]naphthyridine and its methyl derivatives also shows stimulating effect on these compounds. The increase of the activity of acid phosphatase by the used of azaaromatic compounds was ca 14% at 0.01% concentration and ca 15% at 0.01% concentration (average of action of A–C and average of growth phases).

The analysis of experimental results shows that the contents of chlorophyll a and b, and total chlorophyll (a+b) in leaves of celery slightly increase with its physiological age. This regularity concerns so the control plants as well as the plants treated with benzo[h]naphthyridines (Table II). It was observed that A–C change essentially the contents of chlorophyll a and b as well as that of total chlorophyll (a+b).

The results concerning the effect of the azaaromatic compounds (A–C) on the contents of hydrocarbons in leaves of celery are given in Table III. It was observed a stimulating influence of 4,6-benzo[h]naphthyridine and its 1-methyl- and 3-methyl derivatives on the contents of sugars (in general) in celery. An important factor, differentiating the contents of sugars, showed to be the concentration of A–C. The increase of sugars at concentration of 0.01%

II. The influence of 4,6-benzo[h]naphthyridine (A) and its methyl derivatives (B, C) on the contents of chlorophyll a and b, of total chlorophyll (a+b) in leaves celery in different growth phases

Compounds and their contents	Chlorophyll a (mg/g f.m.) growth phases				Chlorophyll b (mg/g f.m.) growth phases				Chlorophyll a+b (mg/g f.m.) growth phases			
	I	II	III	mean	I	II	III	mean	I	II	III	mean
Control	0.160	1.198	1.126	1.161	0.721	0.960	0.872	0.851	1.881	2.158	1.998	2.009
A – 0.01%	1.161	1.216	1.130	1.163	0.720	0.938	0.874	0.844	1.881	2.154	2.004	2.013
B – 0.01%	1.156	1.258	1.140	1.185	0.751	0.969	0.994	0.905	1.907	2.227	2.134	2.089
C – 0.01%	1.149	1.204	1.129	1.161	0.716	1.065	0.881	0.887	1.865	2.269	2.010	2.048
A – 0.1%	1.157	1.283	1.132	1.191	0.725	1.014	0.957	0.899	1.882	2.297	2.089	2.089
B – 0.1%	1.154	1.298	1.145	1.199	0.756	1.045	1.000	0.954	1.910	2.343	2.145	2.133
C – 0.1%	1.190	1.278	1.138	1.202	0.841	1.071	1.007	0.973	2.031	2.349	2.145	2.175
$LSD_{0.05}$	for compounds – 0.009				for compounds – 0.006				for compounds – 0.013			
$LSD_{0.05}$	for phases – 0.013				for phases – 0.009				for phases – 0.015			

III. The influence of 4,6-benzo[h]naphthyridine (A) and its methyl derivatives (B, C) on the contents of vitamin C (mg/kg) and of sugars (% dry mass) in leaves of celery in different growth phases

Compounds and their contents	Vitamin C (mg/kg) growth phases				Sugars (% d.m.) growth phases			
	I	II	III	mean	I	II	III	mean
Control	616.0	742.6	897.6	752.4	0.920	1.630	2.154	1.568
A – 0.01%	783.2	844.8	1034.0	887.3	1.000	1.811	3.220	2.010
B – 0.01%	633.6	774.4	1020.8	809.6	1.010	2.071	3.467	2.183
C – 0.01%	770.0	765.6	792.0	775.9	1.140	1.782	3.008	1.977
A – 0.1%	748.0	840.4	1060.4	882.9	0.930	1.660	2.946	1.845
B – 0.1%	602.8	633.6	695.2	643.9	0.920	1.730	2.852	1.834
C – 0.1%	567.6	673.2	717.2	652.7	0.950	1.650	2.269	1.623
$LSD_{0.05}$	for compounds – 12.9				for compounds – 0.057			
$LSD_{0.05}$	for phases – 19.8				for phases – 0.087			

(average of A–C, average of growth phases) is 31%, and at 0.1% concentration it is only 13%.

The investigated azaaromatics show also as advantageous effect on the contents of vitamin C in celery in its all growth phases (Table III). It was found that here A had the most stimulating influence, the increase of the contents of L-ascorbic acid independent on the concentration of A was ca. 15% as compared with the control plants. The methyl derivatives of 4-6--benzo[h]naphthyridine (compounds B and C) did not show so high influence on vitamin C contents.

DISCUSSION

In order to determine an extent of interference of biologically active compounds into physiological and biochemical processes occurring in plants, at present very often the changes of the enzymic activity of peroxidase, catalase and acid phosphatase are investigated; it is connected with the role these enzymes play in the development (Cañal et al., 1988; De Felipe et al., 1988; Murumcar, Chavan, 1987; Upadhyaya et al., 1985).

Chemical compounds influence in various manner the enzymic activity; the study of benzo[h]naphthyridines have shown their stimulating effect on the peroxidase and catalase activities in the white cabbage and the leek (Herman, 1993).

In our present work we have established that 4,6-benzo[h]naphthyridine, 1-methyl- and 3-methyl-4,6-benzo[h]naphthyridines (heterocyclic, azaaromatic compounds) increase efficiently the peroxidase, catalase and acid phosphatase activities in celery leaves as compared with those in the control plants. The degree of changes of enzymic activity in celery is dependent on its development phase and on the kind and concentration of the used azaaromatic compounds.

The obtained results indicate, that the influence of investigated compounds on the enzymic activity of plants is dependent on their structure; a confirmation may be the results of the study of kinetine and benzyladenine (Kar, Mishra, 1976; Kuroda et al., 1990; Reddy et al., 1985) which are heterocyclic aromatic growth regulators. The above authors suggest that nitrogen and oxygen atoms, present in molecules of these compounds, influence their chemical and biological properties. The mentioned work has shown that due to the increase of peroxidase and catalase activities by kinetine and benzyladenine the ageing process of plants is retarded.

The rise of peroxidase activity along with the simultaneous lowering of the catalase activity with the physiological age of plants, what was observed in our experiments so in the control celery as well in that treated with

4,6-benzo[h]naphthyridine and its derivatives, is held by numerous authors as a symptom of the ageing of plants (Braber, 1980; Kar, Mishra, 1976; Omran, 1980; Upadhyaya et al., 1985).

The increase of the acid phosphatase activity in plants treated with azaaromatic compounds may be connected with acceleration of growth of plants (Angosto et al., 1988; Grapelli, Rossi, 1981).

The nutritive value of agricultural plants is dependent, among others, on the yield of photosynthetic process, therefore the estimation of the total contents of assimilation dyes, and especially of chlorophyll is at present very often applied in investigations of the influence of various external agents, including pesticides and other biologically active compounds on the quality of these plants (Carrol et al., 1988; Grumbach, 1982; Kar, Mishra, 1976).

Chlorophyll is an indicator of the development and ageing of plants (Kar, 1986; Kuroda et al., 1990; Reddy et al., 1985; Upadhyaya et al., 1985). In our experiment a small increase of the content of total chlorophyll (a+b) in celery leaves treated with azaaromatic components was observed.

The stimulating effect of azaaromatics on the contents of chlorophyll along with the simultaneous rise of the activity of peroxidase and catalase may suggest that these substances retard the ageing process of plants in similar way as growth retardants, used in agriculture (Kar, Mishra, 1976; Kuroda et al., 1990; Reddy et al., 1985), this fact being probably connected with their azaaromatic character.

The level of hydrocarbons is directly bound with the chlorophyll contents (Davis et al., 1988; Pharr, Sox, 1984; Qouta et al., 1991). Chinoy (1984) describes the responsibility of assimilation dyes for the accumulation of ascorbic acid.

This activity of applied substances is dependent on a numerous factors, the kind, the species and the variety of plants as well as their physiological state and development phase; also the kind and concentration of used compound and other environmental agents should be taken into account (Murawa, Adomas, 1990; Schaller et al., 1991). On the other hand, the results of examination of growth regulators show definitely their stimulating activity on the contents of hydrocarbons and of vitamin C (Salama, Avadallo, 1989; Sturgis, Ruber, 1982).

The used azaaromatics compounds showed also the stimulating effect on the level of vitamin C and sugars. A confirmation of an advantageous influence of 4,6-benzo[h]naphthyridine and its methyl derivatives on the contents of hydrocarbons and of vitamin C are our former studies concerning the effect of benzo[h]naphthyridines and nitrobenzo[h]naphthyridines on the level of these components in chosen vegetables (Gurgul et al., 1987; Herman, 1993).

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GURGUL, E. – HERMAN, B. – BICZAK, R. – ŚLIWA, W. (Vysoká škola pedagogická, Institut chemie, katedra biochemie a katedra organické chemie, Częstochowa, Polsko):

Vliv 4,6-benzo[h]naphthyridinu a jeho metylových derivátů na enzymovou aktivitu a chemické složení celeru (*Apium graveolans* L. var. *dulce* Pers.) v jeho fázích růstu.

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V nádobových pokusech byl zkoumán vliv 4,6-benzo[h]naphthyridinu a jeho derivátů 1-methyl a 3-methyl-4,6,benzo[h]naphthyridinu na aktivitu peroxidázy a kyse-

lé fosfatázy, jakož i na obsah chlorofylu, cukrů (obecně) a vitaminu C u celeru (*Apium graveolans* L. var. *dulce* Pers.).

Biologicky aktivní azaaromatické sloučeniny, které byly použity v pokusu, způsobily změny enzymové aktivity a biochemického složení v celerových listech.

azaaromatické sloučeniny; celer; peroxidáza; kyselá fosfatáza; chlorofyl; cukry; vitamin C

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