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BRIEF COMMUNICATION

Effect of 1,10-phenanthroline on peroxidase and catalase activity and chlorophyll, sugar, and ascorbic acid contents

B. HERMAN, R. BICZAK and E. GURGUL

Biochemistry Department, Institute of Chemistry, Pedagogical University of Częstochowa, Armii Krajowej 13/15, 42 200 Częstochowa, Poland

Abstract

Changes in the peroxidase (EC 1.11.1.7) and catalase (EC 1.11.1.6) activities, and total chlorophyll, soluble sugars, and ascorbic acid contents of leek leaves treated with the herbicide 1,10-phenanthroline (Phe) in concentrations 0.5, 2.5, 5.0, 7.5, and 10.0 mM have been determined. Plants treated with Phe were characterised by a higher activity of peroxidase and a lower activity of catalase and lower contents of chlorophyll, soluble sugars, and ascorbic acid as compared to non-treated plants.

Additional key words: *Allium ampeloprasum*, enzyme activity, herbicides, leek.

1,10-Phenanthroline (Phe) is a heterocyclic nitrogen compound belonging to diazophenantrens, exhibiting high biological activity (Śliwa 1978). The herbicidal activity of Phe is determined by the presence of two nitrogen atoms and their location in positions 1 and 10 (Nandihalli and Rebeiz 1991). 1,10-Phenanthroline, similarly as other aromatic compounds belongs to metal chelators, therefore its action includes that on chlorophyll biosynthesis (Averina *et al.* 1989, Kittsteiner *et al.* 1991, Mostowska 1992). The strong effect of 1,10-phenanthroline on chlorophyll biosynthesis may lead to a change in the photosynthesis rate (Mostowska *et al.* 1991). Pesticides, may also influence the contents of proteins, sugars, vitamins and change in ezymatic activities. The present work was intended to examine the changes in the activity of catalase and peroxidase and the chlorophyll, soluble sugars, and ascorbic acid contents of leek leaves under the influence of 1,10-phenanthroline during the vegetation period.

Leek plants (*Allium ampeloprasum* L. ssp. *porrum* J. Gay) were grown in plastic pots of a capacity of 7 dm^3 each, filled with brown soil having pH 6.8; containing

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Fax: (+034) 3615154, e-mail: b.herman@wsp.czest.pl

1.6 % of humus, and 140 mg dm^{-3}(soil) N, 120 mg dm^{-3}(soil) P_2O_5, 180 mg dm^{-3}(soil) K_2O, and 70 mg dm^{-3}(soil) MgO. The humidity of the substrate was kept constant - 70 % of the field capacity. Two weeks after the leek seedling had been planted into the pots, the plants were sprayed with 1.10-phenanthroline solutions in concentrations 0.5, 2.5, 5.0, 7.5 and 10.0 mM, using 3 cm^3 of the solution in each concentration per plant. Samples for determination of peroxidase and catalase activities and chlorophyll, soluble sugars, and ascorbic acid contents were taken three times in 2 to 3-week periods, starting on the 30th June. For chemical analyses, three samples of each combination composed of 20 fully grown leaves were taken. In the fresh plant material, the total chlorophyll content was assayed by the spectrophotometric method (Bruinsma 1963), the ascorbic acid content was determined by Tillmans' method, and the soluble sugars in dry mass were analysed by Luff Schoorl's method (for detail see Rutkowska 1981). Extracts were prepared by homogenising the fresh tissue in cooled phosphate buffer with an accurately established pH for a particular enzyme (Gurgul 1982). The activity of peroxidase was determined with *o*-dianisidine as hydrogen donor (Gardiner and Cleland 1974). Absorbance was measured at 450 nm in 15 s intervals for a 3 min period on a spetrophotometer and results were calculated as decrease in absorbance. The activity of catalase was determined as the amount of H_2O_2 decomposed in time (Bergmeyer 1963). The obtained results were subjected to statistical analysis. For chlorophyll, sugars, and ascorbic acid, confidence intervals were determined by Tukey's method, and for catalase and peroxidase activities standard deviations of the means were calculated. To describe the relationship between enzyme activities and phenanthroline concentrations, a linear function was applied.

1,10-Phenanthroline (Phe), in the concentration range of 0.5 to 1.0 mM, changed the total chlorophyll, soluble sugars and ascorbic acid contents of leek leaves (Table 1). During vegetation period a decrease in the total chlorophyll content was

Table 1. The influence of 1,10-phenanthroline on the contents of total chlorophyll [mg g^{-1}(f.m.)], soluble sugars [mg g^{-1}(d.m.)], and ascorbic acid [mg g^{-1}(f.m.)] in leek leaves (determinations 14, 35, and 56 d after application).

Phe	Chlorophyll			Sugars			Ascorbic acid		
[mM]	14 d	35 d	56 d	14 d	35 d	56 d	14 d	35 d	56 d
0	1.79	1.48	1.23	58.3	64.1	86.2	0.547	0.436	0.415
0.5	1.97	1.64	1.36	52.9	59.7	81.6	0.521	0.422	0.363
2.5	1.85	1.52	1.25	48.0	57.2	76.4	0.490	0.379	0.293
5.0	1.75	1.44	1.21	47.2	53.3	71.8	0.472	0.349	0.275
7.5	1.61	1.39	1.14	44.7	51.5	67.7	0.380	0.344	0.263
10.0	1.50	1.26	1.10	45.8	52.0	62.8	0.343	0.330	0.251
$LSD_{0.05}$			0.01			1.7			0.016

observed both in the control plants and in those treated with Phe. At two lower concentrations, Phe increased the chlorophyll content by 11 % at the concentration of 0.5 mM, and by 3 % for the concentration of 2.5 mM in relation to the control. The

higher Phe concentrations (5.0, 7.5, and 10.0 mM) decreased the chlorophyll contents of leek leaves, for the 10.0 mM Phe concentration this decrease being approximately 14 %. The concentration of soluble sugars in leek leaves grew with the age of the plants. During the entire vegetation period, the plants treated with Phe were characterised by lower sugar contents as compared to the control plants. A systematic decrease of the sugar content in leek leaves was observed with increasing Phe concentration, the degree of the changes being similar for all dates of analyses. The performed investigations showed also a decrease in the accumulation of ascorbic acid in leek leaves treated with Phe, with a simultaneous decrease in the concentration of this component with the plant age. For the highest concentrations used this being approximately 34 %. Phe caused also very substantial changes in the activities of peroxidase and catalase (Fig. 1). At the same time, an increase in the peroxidase

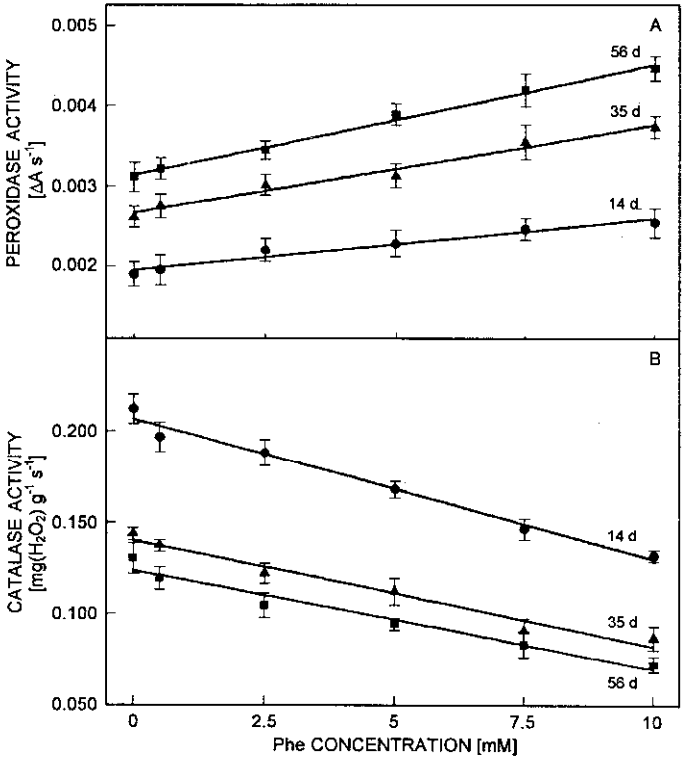

Fig. 1. Relationship between peroxidase (*A*) and catalase (*B*) activities in leek leaves and concentration of 1,10-phenanthroline (14, 35, and 56 d after the treatment). The *vertical bars* represent the standard deviation of the mean. Linear regression equations and correlation coefficients:

	Peroxidase		Catalase	
14 d	$y = 0.00195 + 0.00006x$	$r = 0.96$	$y = 0.20652 - 0.00765x$	$r = 0.98$
35 d	$y = 0.00267 + 0.00011x$	$r = 0.98$	$y = 0.14017 - 0.00582x$	$r = 0.97$
56 d	$y = 0.00314 + 0.00013x$	$r = 0.99$	$y = 0.12349 - 0.00539x$	$r = 0.96$

activity and a decrease in the catalase activity were found with the plant age. Leek leaves sprayed with Phe were characterised by increased peroxidase activity and decreased catalase activity in relation to non-treated plants. The changes in the enzymatic activities were dependent more on plant age than on herbicide concentratration. Thus Phe might accelerate the ageing of plants. A similar correlation between the activities of these enzymes, as a result of using a herbicide - isoproturon causing the premature ageing of the first leaves, is reported by De Felipe *et al.* (1988). The peroxidase activity increase and the catalase activity decrease are reliable indicators of leaf tissue ageing (Braber 1980, Kar and Mishra 1976, Kuroda *et al.* 1990, Reddy *et al.* 1985, Upadhyaya *et al.* 1985). Another characteristic symptom of plant ageing is the chlorophyll degradation (Elstner and Osswald 1980, Kar 1986, Kuroda *et al.* 1990, Reddy *et al.* 1985). The decrease in the total chlorophyll contents of leek leaves under the effect of Phe in higher concentrations, seems to confirm the suggestion that this compound accelerates the ageing in higher plants. The decrease in the chlorophyll contents of plant leaves as the result of Phe action has already been reported (Kittsteiner *et al.* 1991, Mostowska *et al.* 1991, 1992).

References

Averina, N.G., Shalygo, N.V., Yaronskaya, E.B.: Effect of glutamic acid and 1,10-phenanthroline on the accumulation of chlorophyll precursors in green *Phaseolus* leaves. - Photosynthetica **23**: 383-385, 1989.

Bergmeyer, H.U.: Methods of Enzymatic Analysis. - Academic Press, New York 1963.

Braber, J.M.: Catalase and peroxidase in primary bean leaves during development and senescence. - Z. Pflanzenphysiol. **97**: 135-144, 1980.

Bruinsma, J.: The quantitative analysis of chlorophylls *a* and *b* in plant extracts. - Photochem. Photobiol. **2**: 241-249, 1963.

De Felipe, M.R., Lucas, M.M., Pozuelo, J.M.: Cytochemical study of catalase and peroxidase by auxin and abscisic acid during senescence of *Rhoeo* leaf sections. - Plant Physiol. **46**: 806-811, 1988.

Elstner, E.F., Osswald, W.: Chlorophyll photobleaching and ethane production in dichlorophenyl-dimethylurea-(DCMU) or paraquat-treated *Euglena gracilis* cells. - Z. Naturforsch. **35**: 129-135, 1980.

Gardiner, M.G., Cleland, R.: Peroxidase changes during the cessation of elongation in *Pisum sativum* stems. - Phytochemistry **13**: 1095-1098, 1974.

Gurgul, E.: [Effects of irrigation and fertilization by nitrogen and magnesium on activity of some enzymes and yield as well as chemical composition of red cabbage and Italian cabbage.] - Akad. Roln. Szczecin Rozprawa **84**: 26-52, 1982. [In Pol.]

Kar, M.: The effect of photoperiod on chlorophyll loss and lipid peroxidation in exised senescing rice leaves. - J. Plant Physiol. **123**: 389-393, 1986.

Kar, M., Mishra, D.: Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. - Plant Physiol. **57**: 315-319, 1976.

Kittsteiner, U., Mostowska, A., Rüdiger, W.: The greening in cress seedlings. I. Pigment accumulation and ultrastructure after application of 5-aminolevulinate and complexing agents. - Physiol. Plant. **81**: 139-147, 1991.

Kuroda, M., Ozawa, T., Imagawa, H.: Changes in chloroplast peroxidase activities in relation to chlorophyll loss in barley leaf segments. - Physiol. Plant. **80**: 555-560, 1990.

Mostowska, A.: Photodynamic herbicides: their influence on the development and structure of photosynthetic apparatus. - Photosynthetica **26**: 19-31, 1992.

Mostowska, A., Fiett, D., Parys, E.: Influence of 1,10-phenenthroline, a photodynamic herbicide, on the ultrastructure of mesophyll cells and photosynthetic activity in greening pea seedlings. - Environ. exp. Bot. **31**: 385-395, 1991.

Mostowska, A., Kittsteiner, U., Rüdiger, W.: Effect of 1,10-phenanthroline on ultrastructure of pea leaves. - Protoplasma **161**: 23-30, 1991.

Nandihalli, U.B., Rebeiz, C.A.: Photodynamic herbicides. 9. Structure activity study of substituted 1,10-phenanthrolines as potent photodynamic herbicide modulators. - Pesticide Biochem. Physiol. **40**: 27-46, 1991.

Reddy, K.R., Kumar, K.B., Subhani, S.M., Khan, P.A.: Effect of light and benzyladenine on dark-treated growing rice leaves (*Oryza sativa*). I. Changes in chlorophyll content and catalase activity. - Physiol. Plant. **63**: 79-86, 1985.

Rutkowska, U.: Wybrane Metody Badania Składu i Wartości Odżywczej Żywności. [Some Testing Methods for Evaluating of Food Composition and its Nutritional Value.] - PZWL, Warszawa 1981. [In Pol.]

Śliwa, W.: Badania nad benzo[h]naftyrydynami. [Study of the benzo[h]naphthyridines.] - Prace Nauk. Politechnika Wrocławska, Instytut Chemii Organicznej i Fizycznej, Seria: Studia i Materiały, Wrocław 1978. [In Pol.]

Upadhyaya, A., Sankhla, D., Davis, T.D., Sankhla, N., Smith, B.N.: Effect of paclobutrazol on the activities of some enzymes of activated oxygen metabolism and lipid peroxidation in senescing soybean leaves. - J. Plant Physiol. **121**: 453-461, 1985.