Cent. Eur. J. Biol. • 7(6) • 2012 • 1101-1108
DOI: 10.2478/s11535-012-0092-6

VERSITA

Central European Journal of **Biology**

Foliar and root treatments of cucumber with potassium naphthenate: Antioxidative responses

Research Article

Slavko Kevrešan[1,*], Ivana Maksimović[1], Boris M. Popović[1], Dubravka Štajner[1], Marina Putnik-Delić[1], Boško Borković[1], Ksenija Pavlović[2], Ljubica Grbović[2], Vera Ćirin-Novta[2]

[1]*Faculty of Agriculture, University of Novi Sad, 21000 Novi Sad, Serbia*

[2]*Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, 21000 Novi Sad, Serbia*

Received 27 April 2012; Accepted 24 August 2012

Abstract: This work presents a study of the effect of foliar and root application of low concentrations (0.1–10 μM) of potassium naphthenate on the antioxidative status of cucumber (*Cucumis sativus* L.), assessed for both local and systemic organs. Changes in the contents of proline and glutathione indicate that the treatment of plants with potassium naphthenate can be characterized as a mild abiotic stress. The antioxidative system of cucumber plants is sensitive to such treatment, since organs directly exposed to the chemical showed a decrease in total antioxidant activities and an increase in peroxidation. In the organs that were not directly treated, an increase in the total antioxidative activity was observed only at the lowest naphthenate concentration while at higher concentrations this activity tended to decrease. As far as the activities of antioxidant enzymes (guaiacol peroxidase, superoxide dismutase, catalase) are concerned, the responses observed differed between enzymes for a given treatment, but showed similar trends within treated local and untreated systemic organs.

Keywords: *Potassium naphthenate • Cucumber • Antioxidant system • Response • Foliar treatment • Root treatment*

Abbreviations:

GPx - guaiacol peroxidase;
GSH - reduced glutathione;
LP - lipid peroxidation;
SOD - superoxide dismutase;
CAT - catalase;
FRAP - Ferric Reducing Antioxidant Power;
RT - root treatment;
FT - foliar treatment;
K-naph - potassium naphthenate.

1. Introduction

Naphthenic acids represent a complex mixture of aliphatic and cycloalkyl acids that are found in crude oils, where their contents and composition depend on the source of the oil. In the process of refining, they are undesirable since their corrosive action can be harmful to the plant equipment [1]. Because of the presence of carboxylic group in the molecule, these compounds are soluble in water, and hence they can act as environmental pollutants, which is especially pronounced in the processing of "oil sands" in Canada [2]. This environmental aspect has prompted investigations of the content of naphthenic acids in fresh water, their effect on the plant and animal life, as well as their decomposition under natural conditions, which has been a subject of several reviews [3,4]. However, before they became an environmental concern, these compounds were studied as stimulators of plant growth [5], as their effect is similar to that exhibited by plant hormones of the auxin and giberelline families [6,7]. The separation of total naphthenic acids into particular narrower fractions showed that their hormonal activity depends on their molecular structure [8]. It has been found that these compounds influence the action of numerous plant enzymes such as enzymes of CO_2 fixation [9], respiratory enzymes [10], amylase and

* *E-mail: kevresan@polj.uns.ac.rs*

hexokinase [11], glutamic aminotransferase [12], nitrate reductase [13], as well as nitrogen metabolism [14], phosphorus metabolism [15], and other aspects of plant development [16,17].

Studies of our group have shown that these substances influence plant rooting [18-20], as well as the accumulation of particular ions in the root and aboveground parts of plants [21]. The aim of the present work was to study the effect of the three low concentrations of potassium naphthenate (K-naph) (0.1, 1 and 10 μM) in two application modes (through the root and through the leaves) on the total antioxidant capacity and particular parameters of the antioxidant status of cucumber plants. Data on the effect of naphthenic acids on the antioxidant status of plants are practically lacking in the literature. To our knowledge, only the effect of methyl esters of particular naphthenic acids as free-radical scavengers in *in vitro* conditions has been investigated [22,23].

2. Experimental Procedures

2.1 Naphthenic acids and potassium naphthenate

Naphthenic acids were extracted from the atmospheric gas oil fraction (distillation interval 168-290ºC) of Vojvodina crude oil "Velebit" and characterized by IR spectroscopy, GC-MS analysis and other physico-chemical methods [24]. The measured total acid number of the obtained preparation of purified naphthenic acids was 201 mg g^{-1}, which indicates a high level of purity, as the theoretical value is 214 mg g^{-1}. The analysis of low resolution mass spectra showed that the largest portion in the mixture of naphthenic acids is made up by bicyclic carboxylic acids (34.4%), whereas the shares of other classes of acids are: aliphatic 10.4%, monocyclic 15.3%, tricyclic 24.9%, tetracyclic 9.9% and pentacyclic 5.1%. The measured average molecular mass of naphthenic acids was 262 g mol^{-1}, and this value was used to calculate molar concentrations of the prepared solutions. The 1 mM stock solution of K-naph was prepared by dissolving a necessary amount of purified naphthenic acids in a solution containing an equimolar amount of potassium hydroxide.

2.2 Plant material and treatment with potassium naphthenate

For all experiments seeds of the cucumber cultivar "Tajfun" (Institute of Field and Vegetable Crops, NS seme, Novi Sad) were used. Seeds were surface sterilized and germinated in the dark, at 25°C, on sterilized quartz sand and watered daily with demineralised water. Uniform, 5-day old seedlings were transferred to a half-strength Hoagland solution containing (mM) 2.5 $Ca(NO_3)_2$; 2.5 KNO_3; 1.0 KH_2PO_4; 1.0 $MgSO_4x7H_2O$; and (μM) 23.1 B, 4.6 Mn; 0.38 Zn; 0.16 Cu; 0.052 Mo; 8.95 Fe in the form of Fe(III)-EDTA) [25]. The plants were grown in a greenhouse, under a 12-h photoperiod (irradiance of 200-300 μmol quanta m^{-2} s^{-1}), day/night temperatures of 24±2/15±2°C, and a relative humidity of 65-75%. The nutrient solution was changed every third day and the plants were aerated regularly. After 20 days, the plants were treated with K-naph. One group of plants (n=10) was transferred to the nutrient solution containing 0 (control), 0.1, 1 or 10 μM K-naph (root treatment, RT). After 3 days, the nutrient solution was replaced with a new solution of the same composition, and the plants were analysed seven days after the beginning of the treatment. At the same time, another group of plants was treated with potassium salts of naphthenic acids by spraying the leaves with 1 μM KCl (control), 0.1, 1 or 10 μM K-naph (foliar treatment, FT). Spraying was done with 50 ml of each solution in each treatment. After 3 days, the plants were sprayed again in the same way as the first time, and seven days after the beginning of the treatment the plants were analysed. The experiments were done in three replicates and plant material was stored at -70ºC until it was analyzed.

The concentrations of K-naph used in this study (0.1, 1 and 10 μM), were by 10 and 100 times higher compared to the concentration used in our previous studies [18,20,21,26], but were much lower than the concentrations used in phytotoxicity studies [27,28].

2.3 Extraction procedures

Plant material (1 g) was extracted with 25 ml 70% aqueous ethanol (0.1 M HCl) under 30 min sonication in an ultrasonic bath at ambient temperature. The extract was rapidly vacuum-filtered through a sintered glass funnel and kept refrigerated. This extract was used for total antioxidant power determination by the Ferric Reducing Antioxidant Power (FRAP) method.

For the determination of lipid peroxidation (LP) and antioxidant enzymes, 1 g of plant material was extracted with 50 ml 0.1 M K_2HPO_4 (pH 7.0) under 30 min sonication in an ultrasonic bath at ambient temperature. Reduced glutathione (GSH) was extracted with 5% trichloroacetic acid and free proline with 3% sulphosalicylic acid. After 10 minutes of centrifugation at 4ºC and 10,000x*g*, aliquots of the supernatant were used for enzyme and metabolite determinations as outlined below.

2.4 FRAP

Total antioxidant capacity was estimated according to the FRAP assay [29]. The FRAP reagent was

prepared by mixing acetate buffer (300 mM pH 3.6), 2, 4, 6-tripyridyl-s-triazine reagent (10 mM in 40 mM HCl) and $FeCl_3 \cdot 6H_20$ (20 mM) in the ratio of 3:1:1. A sample of 100 µl was mixed with 3 ml of working FRAP reagent and absorbance (593 nm) was measured 4 minutes after vortexing. The test was performed at 37°C. The FRAP value was calculated using the following formula:

FRAP value = ΔAsample (0-4 min)/ΔAstandard (0-4 min)

The 100 µM Fe^{2+} solution was used as a standard; 1 FRAP unit = 100 µM Fe^{2+}

Total antioxidant capacity was expressed in FRAP units.

2.5 Lipid peroxidation

Lipid peroxidation was estimated based on the reactivity of thiobarbituric acid. Samples were evaluated for malondialdehyde production using a spectrophotometric assay. The extinction coefficient at 532 nm of 153,000 mol^{-1} cm^{-1} for the chromophore was used to calculate the malondialdehyde -like thiobarbituric acid complex produced. The colour intensity of the malondialdehyde – thiobarbituric acid complex in the supernatant was measured by its absorbance at 532 nm [30].

2.6 Antioxidant enzymes

The superoxide dismutase (SOD) activity was assayed according to Giannopolitis and Ries [31], by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitro-blue tetrazolium. Glass test tubes containing the mixture were immersed in a bath at 25°C and illuminated with a fluorescent lamp (Philips MLL 5000W). Identical tubes, which were not illuminated, served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm. One unit of SOD was defined as the enzyme activity which inhibited the photoreduction of nitro-blue tetrazolium to blue formazan by 50%, and SOD activity of the extracts was expressed as SOD units per mg of protein.

The guaiacol peroxidase (GPx) activity was measured following the method of Kato and Shimizu [32]. The activity was calculated using the extinction coefficient of 26.6 mM^{-1} cm^{-1} at 470 nm for oxidized tetraguiacol polymer. One unit of GPx activity was defined as the calculated consumption of 1 µmol of H_2O_2 min^{-1} mg $protein^{-1}$.

Total catalase (CAT) activity was determined spectrophotometrically by following the decline of A_{240} due to the consumption of H_2O_2 [33]. One unit of CAT activity decomposes one micromole of hydrogen peroxide per minute at 25°C, pH 7.0. The results were expressed per mg protein.

2.7 Contents of reduced GSH, proline and soluble proteins

The content of GSH was determined with the Ellman reagent at 412 nm, following the procedure of Punitha and Rajasekaran [34]. The concentration of free proline in the leaves and roots was assessed using Bates' methodology [35]. Soluble proteins were determined by the Bradford method [36].

2.8 Statistics and interpretation of results

Statistical significance was tested using a one-way Anova followed by comparisons of means using Duncan's multiple range test ($P<0.05$). Correlation analysis and Anova were done using the statistical software Statistica version 10, Statsoft. Because of the great differences in the absolute values of the particular investigated parameters and for the sake of their mutual comparison, the results were expressed as the percentage of the control.

3. Results

None of the two treatments influenced the plant growth, *i.e.* no significant changes of dry mass were observed between samples (data not shown). There were also no pronounced phenotypic differences between samples – control and treated plants looked similar, although based on the subjective evaluation, the treated plants looked more luxuriant.

In the organs that were directly exposed, a decrease in FRAP was observed, which was accompanied by an increase in LP (Figure 1). The responses in the organs that were not directly exposed to the treatment were observed as an increase in FRAP at the lowest concentration of K-naph (0.1 µM), followed by its decrease at higher concentrations, by about 40% compared to the control. On the other hand, an overall systemic decrease in LP was observed, and this was more pronounced for leaves (by about 40%) than for roots (by about 20%) (Figure 1).

The connectedness between the changes of FRAP and LP is seen from the high value of the coefficient of correlation between these two quantities for root in the RT, which is -0.9601 ($P<0.05$), whereas for the leaves in FT it is -0.7261 ($P<0.05$). The similarity of the responses in the organs that were not directly exposed to the treatment is seen from the coefficients of correlation between the changes in FRAP and LP with the concentration of K-naph, *i.e.* in the leaves in RT and in the root in FT, which are 0.9811 ($P<0.05$) and 0.8478 ($P<0.05$), respectively.

In the FT, a significant increase in the activity was observed for all three measured antioxidant enzymes in

the leaves at the highest concentration of K-naph. The changes in the activity of GPx in the root are similar to those in the leaves, whereas the activities of SOD and CAT show a decrease with increased concentrations of K-naph (Figure 2).

In the organs that were not directly exposed to the treatment, the responses were quite similar with a decrease in the activity of CAT and an increase in the activity of SOD. As for GPx, a mild decrease in the leaves and a mild increase in the root are observed, and the highest concentration of K-naph caused an abrupt increase in the activity of this enzyme (by about 2.5 times) in both organs (Figure 2). The similarity of the responses in the organs that were not directly exposed to the treatment is indicated by the coefficients of correlation for the dependence of the changes in the activities of SOD, GPx and CAT on the concentration of K-naph in the two types of treatment, with respective values of 0.8562 ($P<0.05$), 0.9755 ($P<0.05$), and 0.6768 ($P<0.05$).

Concerning the content of free proline in dependence of the concentration of K-naph, a complex pattern of change was observed in both the roots and leaves in the RT with a significant decrease at the lowest and intermediate concentrations of K-naph. In the FT, a significant decrease of proline in the root was observed at the lowest and intermediate concentrations of K-naph, whereas the content in the leaves directly exposed to treatment showed an abrupt increase at the intermediate K-naph concentration (Figure 3). A significant increase in the content of glutathione was observed only in the leaves in both FT and RT, but the increase was significantly more pronounced when the leaves were directly exposed to the treatment (Figure 3).

4. Discussion

The obtained results can be discussed from two different angles. The first is looking at the effect of different concentrations of K-naph on the antioxidant and stress parameters in the cucumber plants, while the second one is a comparison of local *versus* systemic responses of the treatment.

Although the scavenging activity of naphthenic acid esters has been demonstrated in *in vitro* conditions [22,23], the obtained results show that K-naph in the applied concentrations does not stimulate total antioxidant

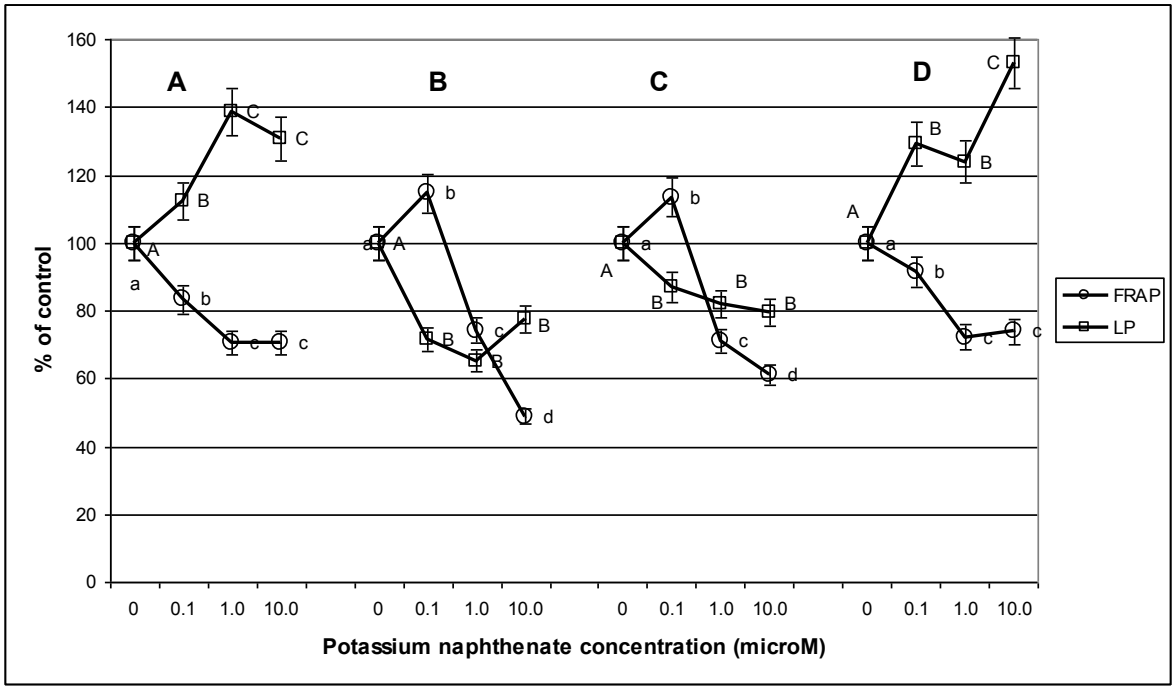

Figure 1. Dependence of Ferric reducing antioxidant power (FRAP) and lipid peroxidation (LP) in the leaves and roots of cucumber plants on the concentration of potassium naphthenate in the root treatment and the foliar treatment. A – root treatment, roots; B – root treatment, leaves; C – foliar treatment, roots; B – foliar treatment, leaves. Bars represent standard deviations (n=9). Within the same treatment, with the different concentrations of potassium naphthenate, the values followed by different letters (capital letters for LP and lower-case letters for FRAP) are significantly different (Duncan's test, $P<0.05$). Control values for FRAP were: 0.72 and 0.79 (in roots, following the root and leaf treatment, respectively), and 5.17 and 4.71 (in leaves, following the root and leaf treatment, respectively) FRAP units (100 μM Fe^{2+}) and for LP: 6.55 and 8.63 (in roots, following the root and leaf treatment, respectively), and 23.587 and 11.26 (in leaves, following the root and leaf treatment, respectively) nmol MDA mg^{-1} protein.

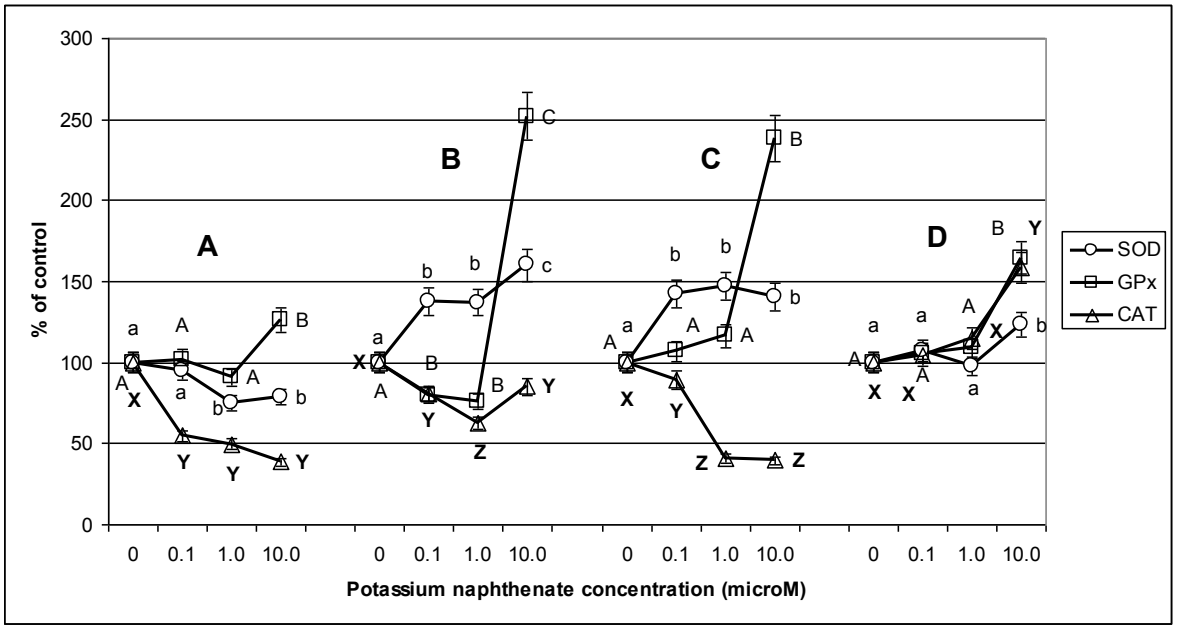

Figure 2. Dependence of the activities of antioxidant enzymes in the leaves and roots of cucumber plants on the concentration of potassium naphthenate in the root treatment and the foliar treatment. A – root treatment, roots; B – root treatment, leaves; C – foliar treatment, roots; B – foliar treatment, leaves. Bars represent standard deviations (n=9). Within the same treatments, with different concentrations of potassium naphthenate, the values followed by different letters (capital letters for guiacol-peroxidase (GPx), lower-case letters for superoxide-dismutase (SOD), and X,Y,Z for catalase (CAT)) are significantly different (Duncan's test, $P<0.05$). Control values for GPx were: 131.32 and 92.39 (in roots, following the root and leaf treatment, respectively) and 38.85 and 33.08 (in leaves, following the root and leaf treatment, respectively); for SOD: 63.81 and 47.46 (in roots, following the root and leaf treatment, respectively) and 63.43 and 52.35 (in leaves, following the root and leaf treatment, respectively); and for CAT: 0.0114 and 0.0116 (in roots, following the root and leaf treatment, respectively) and 0.161 and 0.0956 (in leaves, following the root and leaf treatment, respectively) expressed by U mg^{-1} protein.

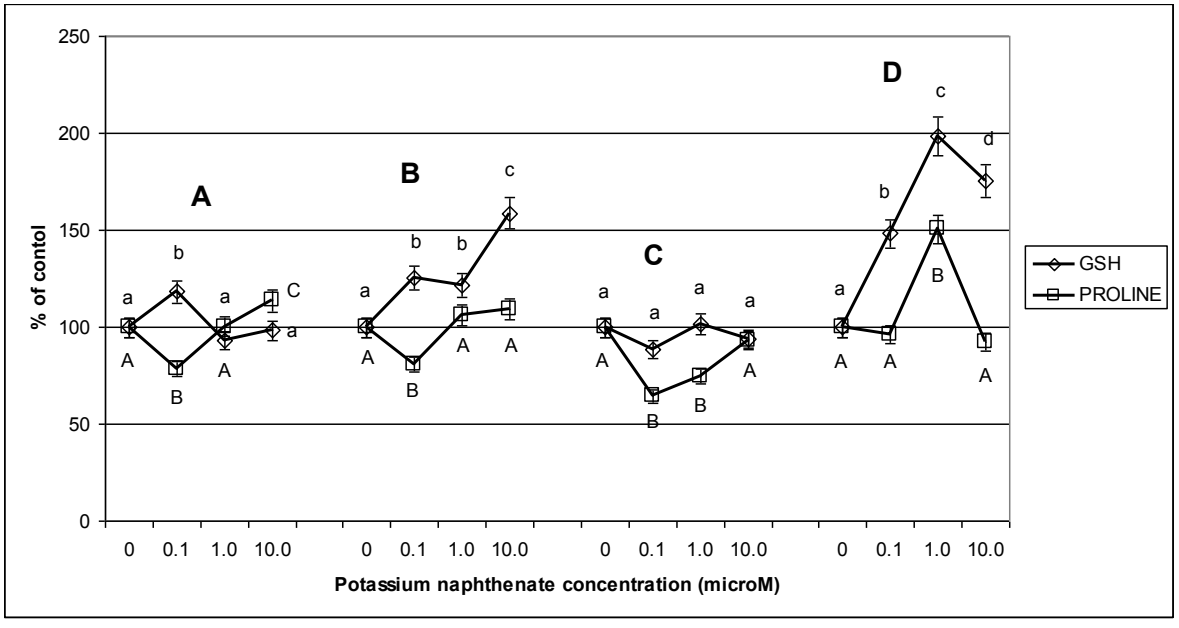

Figure 3. Dependence of the contents of glutathione (GSH) and free proline in the leaves and roots of cucumber plants on the concentration of potassium naphthenate in the root treatment and the foliar treatment. Bars represent standard deviations (n=9). A – root treatment, roots; B – root treatment, leaves; C – foliar treatment, roots; B – foliar treatment, leaves. Within the same treatments, with different concentrations of potassium naphthenate, the values followed by different letters (capital letters for PROLINE and lower-case letters for GSH) are significantly different (Duncan's test, $P<0.05$). Control values for GSH were: 0.18 and 0.19 (in roots, following the root and leaf treatment, respectively) and 0.26 and 0.22 (in leaves, following the root and leaf treatment, respectively) (leaf) μmol mg^{-1} proteins and for proline: 31.41 and 37.03 (in roots, following the root and leaf treatment, respectively), and 33.63 and 41.29 (in leaves, following the root and leaf treatment, respectively).

activity (expressed *via* the FRAP) of cucumber plants with the exception of organs systemically treated with 0.1 μM solution of K-naph. The latter treatment even reduces this activity to a certain extent. In view of the fact that there are no data in the literature about the influence of naphthenates on the antioxidant ability of plants we can only say that the obtained results are in agreement with the reported harmful action of these compounds [27], although the concentrations applied in the present work were lower than those used to study the harmful effect of naphthenates. The increase in activity of SOD and the simultaneous decrease in activities of GPx and CAT, observed in the systemic response to K-naph, indicate the possibility of accumulation of H_2O_2 with the increase in the concentration of K-naph. Only at the highest concentration of K-naph (10 μM), a strong activation of GPx takes place, which prevents excessive accumulation of H_2O_2, as this would lead to a strong oxidative stress. This fact indicates that the antioxidant system of cucumber plants is very sensitive to the presence of naphthenate. Taking into account the changes in the contents of proline and GSH as indicators for plant stress [37,38] it can be thought that the applied treatments with K-naph induced a mild abiotic stress to the cucumber plants.

As a consequence of increased environmental pollution, plants are very often exposed to chemicals that are unknown to the plant species, so called xenobiotics. These chemicals, as stress factors, can induce damage to some molecules, mostly caused by oxidation, such as membrane lipids, photosynthetic pigments, proteins or DNA [39]. In view of these findings, the observed increase of the lipid peroxidation in the organs directly exposed to K-naph (Figure 1, A and D), shows that K-naph can act as a xenobiotic.

Very frequently, only one part of the plant is directly exposed to the action of a stress factor. In these cases, in order to retain their integrity and homeostasis, the plants react to the stressor both locally and systemically, most likely through phloem- or xylem-mobile signalling molecules [40-42]. Long-distance signalling in plants can be established by the transport of molecules such as hormones, proteins, and RNA molecules. These signals are then most likely perceived by receptors and transmitted to the nucleus, where transcription factor complexes induce characteristic changes in gene expression [43]. It might be speculated that in the present experiment the treatment with K-naph produced signalling molecules locally, which were then transported systemically to induce very similar responses in those distal organs (Figure 1, B and C).

5. Conclusions

The antioxidant system of cucumber is sensitive to the application of K-naph since the parameters of its antioxidative status change already at a very low concentration of this agent.

The experimental design involving foliar and root treatments of cucumber plants with different concentrations of K-naph resulted in responses (FRAP, LP, and antioxidative enzymes) that depended on the mode of application. In any given treatment, the responses differed between local and systemic organs. Concerning FRAP and LP, the organs that were directly exposed to increasing K-naph concentrations responded in a similar way in both treatments. The systemic responses in distal organs were also similar irrespective of the mode of application.

Acknowledgements

This study was supported by the Ministry of Education and Science of the Republic of Serbia, Project No. 172006 and TR 31036.

References

[1] Babaian-Kibala E., Petersen P.R., Humphries M.J., Corrosion by naphthenic acids in crude oils, Prepr.-Am. Chem. Soc. Div. Pet. Chem., 1998, 3, 106-110
[2] Headley J.V., McMartin D.W., A review of the occurrence and fate of naphthenic acids in aquatic environments, J. Environ. Sci. Health, 2004, Part A 39, 1989-2010
[3] Clemente J.S., Fedorak P.M., A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids, Chemosphere, 2005, 60, 585–600
[4] Headley J.V., Peru K.M., Barrow M.P., Mass spectrometric characterization of naphthenic acids in environmental samples: a review, Mass Spec. Rev., 2009, 28, 121–134
[5] Parups E.V., Naphthenic acids and related compounds as plant growth regulators, Greenhouse Garden Grass, 1969, 8, 15-19
[6] Ćirin-Novta V., Kuhajda K., Kevrešan S., Kandrač J., Radić Lj., Biological activity and structure of natural petroleum acids from lower oil fractions of "Velebit" oil, Acta Period. Technol., 2002, 33, 135-141

[7] Ćirin-Novta V.S., Kuhajda K.N., Kevrešan S.E., Kandrač J.E., Grbović Lj.M., Rodić P.A., Structural and physiological properties of natural petroleum acids from middle oil fractions of "Kelebija" oil, Acta Period. Technol., 2004, 35, 87-93
[8] Grbović Lj., Pavlović K., Prekodravac B., Kuhajda K., Kevrešan S., Popsavin M., et al., Fractionation of complex mixture of naphthenic acids, their characterization and biological activity, J. Serb. Chem. Soc., 2012, 77, 147-157
[9] Wort D.J., Mechanism of plant growth stimulation by naphthenic acid. II. Enzymes of CO2 fixation, CO2 compensation point bean embryo respiration, Plant Physiol., 1976, 58, 82-86
[10] Fattah Q.A., Hussain Z., Bano A., Enhanced activity of two respiratory enzymes in maize leaves following potassium naphthenate application, J. Asiatic Soc., Bangladesh, 1978, 2, 59-62
[11] Fattah Q.A., Hussain Z., Bano A., Effect of potassium naphthenate on amylase and hexokinase activity of maize grains, Bangladesh J. Bot., 1978, 7, 77-82
[12] Hussain Z., Fattah Q.A., Bano A., Glutamic aminotransferase activity in maize leaves as affected by potassium naphthenate treatment, Bangladesh J. Sci. Ind. Res., 1980, 15, 162-164
[13] Bano A., Fattah Q.A., Hussain Z., Relationship between nitrate reductase, nitrogen content, grain yield and protein content of potassium naphthenate treated maize plants. Indian J. Plant Physiol., 1980, 23, 238-243
[14] Wort D.J., Severson J.G., Peirson D.R., Mechanism of plant growth stimulation by naphthenic acid. Effects on nitrogen metabolism of Phaseolus vulgaris L., Plant Physiol., 1973, 52, 162-165
[15] Severson Jr J.G., Effect of naphthenic acid on 32P incorporation in the organs of the bush bean, Phaseolus vulgaris L., Phyton, 1974, 32, 81-87
[16] Ullah M.J., Fattah Q.A., Hossain F., Response of growth, yield attributes and yield to the application of KNap and NAA in cowpea, Bangladesh J. Bot., 2007, 36, 127-132
[17] Fattah Q.A., Wort D.J., Metabolic responses of bush bean plants to naphthenate application, Can. J. Bot., 1970, 48, 861-866
[18] Kevrešan S., Ćirin-Novta V., Vasić D., Kuhajda K., Kandrač J., Petrović N., et al., Effect of naphthenic acids on formation of adventitious roots in sunflower cuttings, Helia, 2003, 26, 75-82
[19] Halmagyi A., Kevrešan S., Kovačević B., Orlović S., Miladinović D., Ćirin-Novta V., et al., Effects of naphthenic acids on rooting of in vitro grown Sequoia sempervirens, Acta Hort., 2010, 885, 139-144
[20] Kevrešan S., Kovačević B., Ćirin–Novta V., Kuhajda K., Kandrač J., Pavlović K., et al., Biochemical changes in cuttings of Robinia pseudoacacia after treatment with naphthenate, J. Serb. Chem. Soc., 2007, 72, 953-959
[21] Kevrešan S., Ćirin-Novta V., Kuhajda K., Kandrač J., Petrović N., Grbović Lj., et al., Effect of low concentration of sodium naphthenate on uptake of some metal ions by soybean plants, Belg. J. Bot., 2005, 138, 11-13
[22] Štajner D., Ćirin-Novta V., Pavlović A., Scavenger properties of synthetic naphthenic methyl esters, Z. Naturforsch., 1998, 53, 871-875
[23] Štajner D., Ćirin-Novta V., Bajin K., Antioxidative and physiological action of naphthenic methyl esters, Oxid. Commun., 2001, 24, 525-522
[24] Ćirin-Novta V.S., Kevrešan S.E., Kuhajda K.N., Kandrač J.E., Grbović Lj.M., Rodić P., Optimization of petroleum acid isolation from lower oil fractions of Vojvodina "Velebit" oil, Acta Period. Technol., 2003, 34, 49-54
[25] Hoagland D.R., Arnon D.I., The water-culture method for growing plants without soil, Calif. Agric. Exp. Stn. Circ., 1950, 347, 1-32
[26] Kevrešan S., Ćirin-Novta V., Kuhajda K., Kandrač J., Petrović N., Grbović Lj., et al., Alleviation of cadmium toxicity by naphthenate treatment, Biol. Plant., 2004, 48, 453-455
[27] Kamaluddin M., Zwiazek J.J., Naphthenic acids inhibit root water transport, gas exchange and leaf growth in aspen (Populus tremuloides) seedlings, Tree Physiol., 2002, 22, 1265-1270
[28] Armstrong S.A., Headley J.V., Peru K.M., Ermida J.J., Phytotoxicity of oil sands naphthenic acids and dissipation from systems planted with emergent aquatic macrophytes, J. Environ. Sci. Health, 2008, 43, 36-42
[29] Benzie I.F.F., Strain J.J., Ferric reducing antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, Methods Enzymol., 1999, 299, 15-27
[30] Ng T.B, Liu F., Wang Z.T., Antioxidative activity of natural products from plants, Life Sci., 2000, 66, 709-723
[31] Giannopolitis C.N., Ries S.K., Superoxide dismutases. I. Occurrence in higher plants, Plant Physiol., 1977, 54, 309-14
[32] Kato M., Shimizu S., Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves; phenolic dependent peroxidative degradation, Can. J. Bot., 1987, 65, 729-735

[33] Srivalli B., Sharma G., Khanna-Chopra R., Antioxidative defence system in an upland rice cultivar subjected to increasing intensity of water stress followed by recovery, Physiol. Plant., 2003, 119, 503-512
[34] Punitha S.C., Rajasekaran M., Antioxidant mediated defence role of Wedelia calendulacea herbal extract against CCl4 induced toxic hepatitis, J. App. Pharm. Sci., 2011, 1, 111-115
[35] Bates L.S., Rapid determination of free proline for water-stress studies, Plant Soil, 1973, 39, 205-207
[36] Bradford M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem., 1976, 72, 248-253
[37] Claussen W., Proline as a measure of stress in tomato plants, Plant Sci., 2005, 168, 241–248
[38] Tausz M., Šircelj H., Grill D., The glutathione system as a stress marker in plant ecophysiology: is a stress-response concept valid?, J. Exp. Bot., 2004, 55, 1955-1962
[39] Ramel F., Sulmon C., Serra A., Gouesbet G., Couée I., Xenobiotic sensing and signalling in higher plants, J. Exp. Bot., 2012, 63, 3999-4014
[40] Yi, H-S., Yang J.W., Ghim S-Y., Ryu C-M., A cry for help from leaf to root, Plant Signal. Behav., 2011, 6, 1192-1194
[41] Bezemer T.M., van Dam N.M., Linking aboveground and belowground interactions via induced plant defences, Trends Ecol. Evol., 2005, 20, 617-624
[42] Heil M, Ton J., Long-distance signalling in plant defence, Trends Plant Sci., 2008, 13, 264-272
[43] Kaufmann K., Smaczniak C, de Vries S., Angenent G.C., Karlova R., Proteomics insights into plant signalling and development, Proteomics, 2011, 11, 744–755