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Plant stress activated by chlorine from disinfectants prepared on the base of sodium hypochlorite

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Abstract

In this study, the phytotoxicity of disinfectants prepared on the base of sodium hypochlorite was determined. For our tests two commercial products, Savo and Dom Amor, as well as 10% NaClO solution were used. While Savo contained only NaClO, Dom Amor contained NaClO and earthworm enzymes. Products on the base of NaClO are used in households for cleaning and disinfection of floors, furniture, sanitary and kitchen equipment. Savo may be used for the disinfection of drinking waters as well. Products with NaClO are also used for bacteria, algae and pathogens reduction in irrigation waters. As a subject, young seedlings of mustard *Sinapis alba* L. were used for the study of chronic toxicity. The observed parameters of the inhibition of roots and shoots growth, dry (DM) and fresh (FM) mass as well as photosynthetic pigments production (chlorophyll a, b, carotenoids) and water content in the plants were determined. The results point out that Dom Amor was the most toxic for *S. alba* seedlings growth and the rank order of the FAC contents for both plant parts was arranged as: Dom Amor > Savo > NaClO. All disinfectants reduced the DM and FM of roots; however, they stimulated biomass production in the shoots. On the basis of the obtained results it could be concluded, that disinfectants stimulated photosynthetic pigments production and reduced water content mainly in the roots. Dom Amor did not significantly reduced the water content in the shoots and for this parameter the following rank orders of inhibition for roots and shoots could be arranged as NaClO > Dom Amor > Savo and NaClO > Savo > Dom Amor, respectively. All commercial products increased chlorophyll a (Chla) and the carotenoids (Car) content in the shoots. As significant increase was confirmed first for Chla whose content in the presence of NaClO at concentration 24 mL/L overextended that in the control by 3.5 times. The rank orders of stimulation for Chla and Car were NaClO > Savo > Dom Amor and Dom Amor > NaClO > Savo, respectively.

Introduction

Chlorine is classified as a plant micronutrient essential for proper plant growth and all crops require it in small quantities. It is important for photosynthesis - acts as co-factor (Hajrasuliha 1980), it is involved in the opening and closing of stomata and plays some important role in osmotic adjustment and plant disease suppression (Slabu *et al.* 2009). It is also important for enzyme activity regulation in the cytoplasm, acts as a counter anion to stabilize membrane potential, and is involved in turgor and pH regulation (Xu *et al.* 2000; White and Broadley 2001). Chlorine

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deficiency evokes chlorosis and necrosis. Necrosis appears along leaves margins and tips, leaves are smaller than usual and plant growth is also reduced (Montero *et al*. 1998; Marschner and Marschner 2012). Symptoms are usually seen first on older leaves. High chloride concentration can reduce the yield (Albacete *et al*. 2008). Crops differ both in their chloride requirements as well as in their tolerance to chloride toxicity.

Since chloride is an anion, it does not adsorb to soil particles and moves readily with the water in the soil. Therefore, water quality and irrigation management are the major factors that affect chloride concentration in soil. Other chloride sources in the soil are some fertilizers. Chloride toxicity is often accompanied or complicated by salinity or infiltration problems which appear when salinity is low. The most common toxicity is from chloride in irrigation water. As chloride is not adsorbed to soil, it moves readily with the soil water and is taken up by the crop, moves in the transpiration steam, and accumulates in the leaves. However, toxic symptoms appear when the chloride content in the leaves achieves 0.3 to 1.0% on dry weight, and sensitivity depends on crop. As presented by Xu *et al*. (2000) and White and Broadley (2001) the critical concentration for chloride toxicity is estimated to be 4-7 mg/L for Cl^- sensitive and 15 – 50 mg/L for Cl^- tolerant species. Chloride transport and exclusion from shoots is correlated with salt tolerance in many species. Crop tolerance to chloride is not nearly so well documented as crop tolerance to salinity, however, its concentration is important for salt tolerance (Tavakkoli *et al*. 2010; Teakle and Tyerman 2010).

Chlorine inputs to soils occur mainly as a result of Cl^- deposition from rainwater, fertilizer applications (KCl), irrigation water, sea spray, dust and air pollution. Cl^- deposition from human activity belongs mainly to irrigation and fertilization. However, some agricultural establishments try using water for irrigation from reservoirs or ponds, such water recycling may disperse plant pathogens into crops (Hong and Moorman 2005). Bush *et al*. (2003) presented that recycled irrigation water from harbors contained substantial densities of pathogens and Hong and Moorman (2005) reported the presence of 17 *Phytophthora* sp., 26 *Pythium* sp., 27 genera of fungi, 8 species of bacteria, 10 viruses and 13 species of plant parasitic nematodes in irrigation water from ponds, rivers, canals, streams, lakes, runoff water, etc. These facts explain why chlorine is used for disinfection of irrigation water (Cayanan *et al*. 2009).

At present, it is widely used in agriculture, chemical, paint- and lime, food, glass, paper, pharmaceutical, synthetic and water disposal industries. In the textile industry, it is used as a bleacher and chlorine compounds could also be used for electrochemical treatment of water contaminated with dyes (Valica and Hostin 2016). Sometimes NaClO is added to industrial waste waters to reduce odor. Hypochlorite can be used to prevent algae and shellfish growth in cooling towers and in water treatment, it is used for water disinfection. In households, it is frequently used for the purification and disinfection of the house. However, water with commercial sodium hypochlorite products released into a water environment has relatively low concentrations enough, strong toxic effects on freshwater invertebrates and bacteria may appear (Fargašová 2017).

By adding sodium hypochlorite to water, hypochlorous acid (HClO) and hypochlorite ions (ClO^-) are formed. The equilibrium of these two forms depends only on water pH (pKa for $HClO/ClO^-$ is ~ 7.4). NaClO in water is gradually depleted for oxidation of inorganic and organic compounds (Mohammadi 2008). However, NaClO is very effective and low cost biocide used for water disinfection (Amin *et al*. 2013), hypochlorite is a highly destructive, selective oxidant that reacts easily with all biomolecules. Moreover, dissolved chlorine dissociated into hypochlorite and hypochlorous ions, which penetrate cell membrane, may result in the formation of genotoxic, mutagenic, and/or carcinogenic disinfection by-products (DPBs) (Sapone *et al*. 2016) and are also associated with adverse reproductive outcomes (Nieuwenhuijsen *et al*. 2000).

As described by White and Broadley (2001), chlorine occurs in the soil predominantly as a Cl^- anion, which does not form complexes readily, and is repelled from predominantly negatively charged mineral surfaces of many soil particles. This fact changes Cl^- movement in the soil and the consequence of its slight adsorption to soil components is not chemically altered by soil organisms. Its movement within the soil is largely determined by water flows. That's why is often used as a tracer for soil water movement.

During our study, attention was focused on free available chlorine – the FAC (mg/L) effect of NaClO and two commercial disinfectant products, prepared on NaClO base, on some physiological processes in crop *Sinapis alba*. Chlorine enters the plants through the roots by a symplastic pathway and is mobile within the plant. Its fluxes across membranes and between tissues limited growth, water translocation in the plant and interferes with photosynthesis through inhibition of photosynthetic pigments production. In an effort to avoid chlorine movement through soils and the assurance of direct FAC effect on plant growth and physiological activity, only water solutions of sodium hypochlorite products were used.

Experimental

Disinfectans

The following commercial products on the base of sodium hypochlorite (NaClO) were investigated with regard to their phytotoxic effects – Savo (produced by Bochemie a.s., Bohumín, Czech Republic) and Dom Amor (produced by BOOS Biologické substancie, Košice, Slovak Republic). Savo contained only NaClO; while Dom Amor contained both NaClO and earthworm enzymes. The content of effective ingredients (CEI) of products did not exceed 5%. The toxicity of sodium hypochlorite (NaClO) (10% solution) was also determined. The active substance of NaClO in water is hypochlorous acid (HClO) and hypochlorite ions (ClO^-). Fresh samples were used for each test due to high chlorine volatility. The concentration of free available chlorine (FAC) of products was determined by the reaction of the tested products with phosphate buffer and potassium iodide, followed by titration of thiosulphate on starch (Añasco *et al.* 2008). The results of the analyses for three tested products are presented in Table 1. However, only free active chlorine concentrations, being the most active and dominant form, are presented here. Doses of the disinfectants used in the tests were decided after preliminary experiments performed in the laboratory according to the corresponding guide (OECD 208 2006).

Table 1. Content of effective ingredients – CEI (%) and the concentration of free available chlorine – FAC (mg/L) in tested substances.

	CEI (%)	FAC (mg/L)
Savo	5	39.90
Dom Amor	5	32.21
NaClO	10	108.86

The dissipation tests were conducted before the experiments due to chlorine volatility. To provide a constant chlorine level, the solutions were replaced every 24 hours. Under these conditions, FAC concentration at the end of the experiments did not decrease below 90%. The typical levels of free chlorine in drinking water ranged from 0.2 – 2.0 mg/L, though regulatory limits allow levels as high as 4.0 mg/L.

Sinapis alba *growth inhibition test*

Mustard *Sinapis alba* L. seeds were germinated in Petri dishes with 17-cm diameter, laboratory filter paper No. 1 disks and plastic nets on the bottom. Nine different concentrations from each sample (Table 2), designed on the base of orientation tests, were prepared in dechlorinated tap water (80 mg/L Ca, 27 mg/L Mg, pH = 7.3±0.05). Each Petri dish contained 50 mL of tested solution and 20 healthy looking and similar size mustard seeds spread on the plastic net situated on the filter paper laid down on the bottom of the dish. As a control, only tap water was used in the same way. Dishes were put in a dark thermostat at 22±1°C and plastic nets with germinated seeds were replaced every 24 hours to fresh tested solutions. After 72 hours the roots and shoots length was measured and IC50 concentrations were calculated by using probit analysis (Fargašová and Lištiaková 2009).

Photosynthetic pigments and water content determination

After 72 hours plastic nets with seedling were transferred to hydroponic dark containers (250 mL) with control or disinfectants solutions, situated in laboratory box with a day-light cycle (16/8), and the temperature was maintained at 23±2 °C. Afterwards, the dishes were shielded from the direct sunlight and the cultivation continued for the next 7 days. During this period, the tested solutions were changed every 24 hours in the same way as was described before to provide a constant chlorine concentration during the entire experiments. During this cultivation shoots were not in direct contact with the disinfectant solutions. After 10 days of growth, the plants were harvested and the roots and shoots were separated. Fresh mass (FM) was weighed immediately after organ separation. The pigment contents (chlorophyll a, b, total carotenoids) were determined in the fresh shoots after extraction in 95% ethanol (w/v) (1 g of fresh shoots per 6 mL of ethanol). Pigment extraction continued until all of the homogenized plant mass was white. After a brief centrifugation (2 min at 2 900 x g), the pigment content was measured spectrophotometrically at 665, 649 and 470 nm in supernantant (Lichtenthaler and Wellburn 1983). Photosynthetic pigments amount was calculated by the following equations:

$$Chla = 13.95\ (A_{665}) - 6.88\ (A_{649})$$
$$Chlb = 24.96\ (A_{649}) - 7.32\ (A_{665})$$
$$Car = [1\,000\ (A_{470}) - 2.05\ (chl\ a) - 114.8\ (chl\ b)]/245$$

(Chla – chlorophyll a concentration, Chlb – chlorophyll b concentration, Car – carotenoids concentration; in μg/mL of plant extract).

Afterwards plant material – roots and shoots, were separately dried at a temperature of 40 °C to a constant mass and then weight. From the obtained values of fresh (FM) and dry mass (DM), the water content was calculated (Drazic and Mihailovic 2005):

$$WC = (FM - DM)/DM$$

(WC – water content, FM – fresh mass, DM – dry mass) in g/g DM of *S. alba* seedling organs and the effect of tested solutions on WC in the roots and shoots was determined as a standardized values of the reference control.

Table 2. Concentrations of Savo, Dom Amore and NaClO used during the tests with *S. alba* seedlings expressed as the FAC concentrations in mg/L and the volume of solution mL/L (used concentrations were selected on the base of preliminary tests).

Compound		Concentration
Savo	mL/L NaClO	2.0; 4.0; 6.0; 8.0; 12.0; 16.0; 24.0; 32.0;
	mg/L FAC	0.08; 0.16; 0.24; 0.32; 0.48; 0.64; 0.96; 1.28;
Dom Amore	mL/L NaClO	2.0; 4.0; 6.0; 8.0; 12.0; 16.0; 24.0; 32.0;
	mg/L FAC	0.064; 0.13; 0.19; 0.26; 0.39; 0.52; 0.77; 1.03;
NaOCl	mL/L NaClO	2.0; 4.0; 6.0; 8.0; 12.0; 16.0; 24.0; 32.0;
	mg/L FAC	0.22; 0.44; 0.65; 0.87; 1.31; 1.74; 2.6; 3.48;

Statistical analysis

All phytotoxicity tests were carried out in triplicate, and they included a control in tap water. Quality control data were considered acceptable according to control charts and other established criteria. The ADSTAT 2.0 statistical software was used for statistical evaluation. A T-test was used to assess the significant difference between the controls and other treatments.

Results and Discussion

All results obtained by the use of the above mentioned methods were evaluated according to an ecological risk assessment framework which implies the examination of risks from natural, human and industrial activities (Fargašová 2016). Experiments with the crop *S. alba* confirmed that chlorine ions are generally toxic to plants' growth at relatively low concentrations and may cause irreversible damage of their development. Free chlorine is often added to irrigation water for algae, fungi, and bacteria destruction. Chlorination is used mainly for surface irrigation water from rivers, canals, reservoirs and ponds, as well as for the prevention of clogged piping by organic material (Cayanan *et al*. 2009; Parke and Fisher 2012). As we presented before, the usage of disinfectant prepared on the base of NaClO is very effective for such prevention, because free chlorine levels which destroy algae are at least 10-times lower than those which reduced plant growth by 50% (Fargašová 2017). The inhibitory concentrations (IC) which reduced roots and shoots growth of mustard by 50% together with their confidence intervals (CI) are introduced in Table 3. From these results, it is evident that the roots of *S. alba* were more sensitive to disinfectants than shoots, and the commercial product Dom Amor enriched with earthworm

Table 3. IC50 concentrations and their 95% confidence intervals (CI) for roots and shoots growth inhibition (values are introduced as volume of compound in mL/L as well as the concentration of free available chlorine in mg/L FAC).

Compound	Roots growth		Shoots growth	
	IC50 ± (CI) (mL/L)	**IC50 ± (CI) (mg/L FAC)**	**IC50 ± (CI) (mL/L)**	**IC50 ± CI) (mg/LFAC)**
Savo	12.68 (12.20 – 13.25)	0.51 (0.49 – 0.53)	19.98 (18.45 – 25.59)	0.80 (0.74 – 1.02)
Dom Amor	6.85 (6.25 – 7.33)	0.22 (0.20 – 0.24)	18.58 (17.32 – 20.38)	0.60 (0.55 – 0.65)
NaClO	5.16 (4.96 – 5.33)	0.56 (0.54 – 0.58)	8.84 (8.65 – 9.05)	0.97 (0.94 – 0.99)

enzymes had the strongest inhibitory effect on both plant parts. For inhibition, the following rank orders for FAC content should be arranged as so: roots growth: Dom Amor > Savo ≥ NaClO; shoots growth: Dom Amor > Savo > NaClO. All tested compounds reduced the growth more for roots than shoots. This might be explained by Greenway and Thomas (1965) conclusion, which assume that the shoots initially contained no Cl^- and therefore, the toxic effects of chlorine don't appear mainly in young seedlings. According to the growth responses of plants of high Cl^- concentration in the environment plants could be divided into four categories. The differences between them are often related to the ability to restrict Cl^- transport to shoots (White and Broadley 2001). Large differences between the chlorine content in various plant parts and its relation to the manifestation toxic effects, was also confirmed (Greenway and Munns 1980) long before. Chlorine, as an essential element, supporting plant growth is taken into the xylem and thereby delivered to the shoots (White and Broadley 2001). There are two pathways for Cl^- anion intake into the xylem – the symplastic (cytoplasmic) and the apoplastic (extracellular). The intake process influences fluxes and accumulation of the chlorine into the plant and its distribution within the plant. Toxic effects of chlorine on plants development were confirmed also by Carrilo *et al*. (1996) to radish and lettuce seedlings after the application of a commercial dioxide product (Hallox) with 2.6 mg/L of active chlorine. Hallox was in this case used for direct irrigation on a soil surface at a level of 1 : 1000. As presented by Cayanan *et al*. (2009) chlorine had no phytotoxic or growth effects on all plants and growth inhibition depends on free active chlorine content. In addition to growth, dry and fresh mass production could also be affected and this was also confirmed by our experiments.

Plant growth is very closely connected with crop production expressed as dry (DM) and fresh (FM) mass production. Both these parameters are very strongly influenced by chlorine fluxes and accumulation within the roots and its translocation into the shoots (White and Broadley 2001). Chlorine intake is closely connected with water uptake and translocation through plants and the

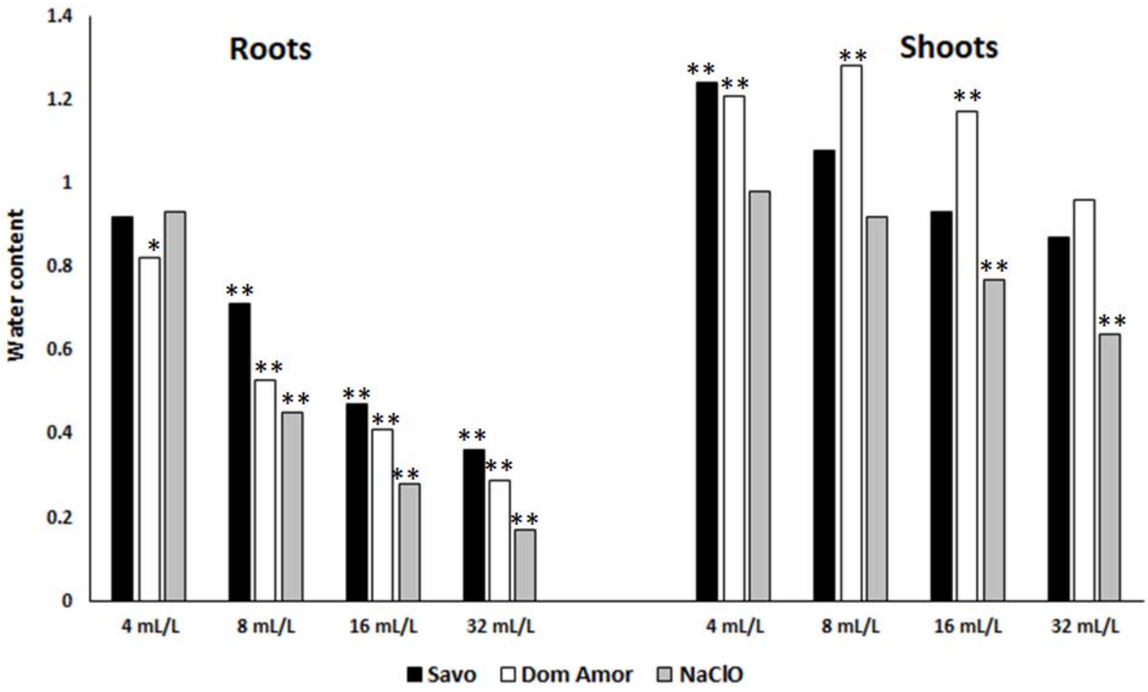


Fig.1. Water content in the roots and shoots of *S. alba* after 10 days' application of Savo, Dom Amor and NaClO disinfectants introduced as standardized values of the reference control (water content in control = 1). Statistical significance: * $P < 0.05$; ** $P < 0.01$.

results from these observations are shown on Fig. 1. The presented results indicate that all products in all used concentrations reduced more water reception by the roots than its translocation into the shoots. Plants try to supply chlorine toxicity by its movement through water into the upper part of the plant and in this way, attempt to reduce chlorine toxicity in the roots which are responsible for nutrients' reception. As presented by White and Broadley (2001), plants support their growth by loading chlorine into the xylem and thereby deliver it to the shoots. This is in accordance with the results we obtained during the determination of water content and dry and fresh mass (Fig. 1, 2). These authors showed that there are two pathways by which anions might reach the xylem: (1) Anions enter root cells through a plasma membrane, are then transferred from cell to cell through plasmodesmatal connections, and are exported across the plasma membrane of cells into the stele; (2) anions are taken extracellularly through the cell wall and water spaces to reach the stele – this is a relatively non-selective process governed by the transport of water through solvent drag. Tested compounds significantly reduced water content only in the roots while the water content in the shoots was stimulated or reduced only slightly. However, Dom Amore increased the water content in the shoots in all of the tested concentrations; NaClO indicated a reduction which did not exceed 64%. The following rank orders of water content reduction could be arranged as so: for roots NaClO > Dom Amor > Savo, for shoots NaClO > Savo > Dom Amor.

Water reception from the solvents with NaClO, including the two commercial disinfectant products Savo and Dom Amor, is very closely connected with dry (DM) and fresh (FM) production by the roots and its translocation to upper plant parts (Fig. 2) From the obtained results, it can be concluded that while DM and FM production of the roots was reduced in the presence of chlorine from all tested compounds, DM and FM of the shoots production was stimulated and this is in accordance with the calculations and equations by White and Broadley (2001), who confirmed the close relation between the root uptake of chlorine and a plant's relative growth and biomass production. As was previously presented, chlorine is an essential micronutrient for higher plants and its minimal requirement for crop growth is 1 g/kg DM. However, high chlorine concentration in tissues can

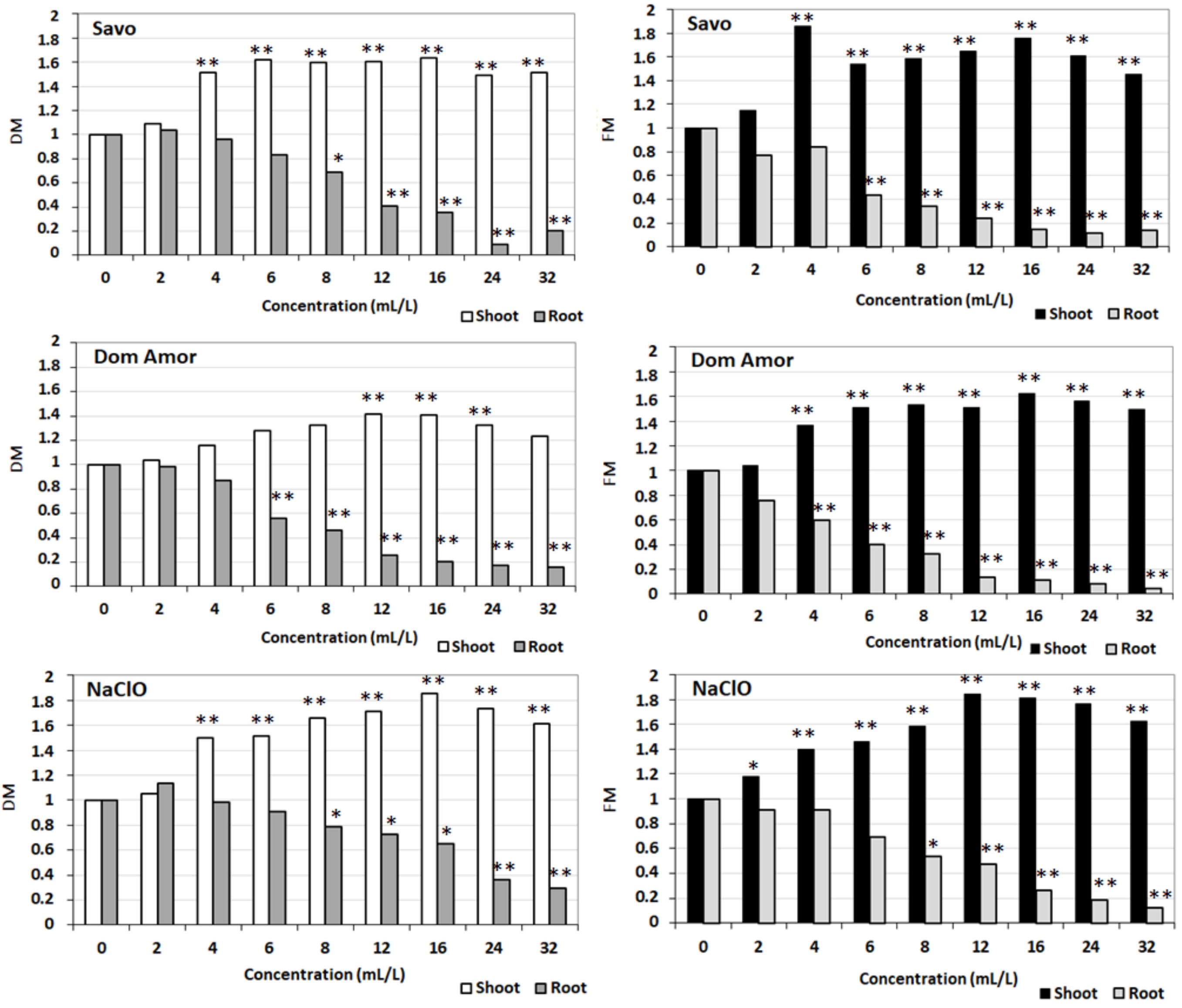
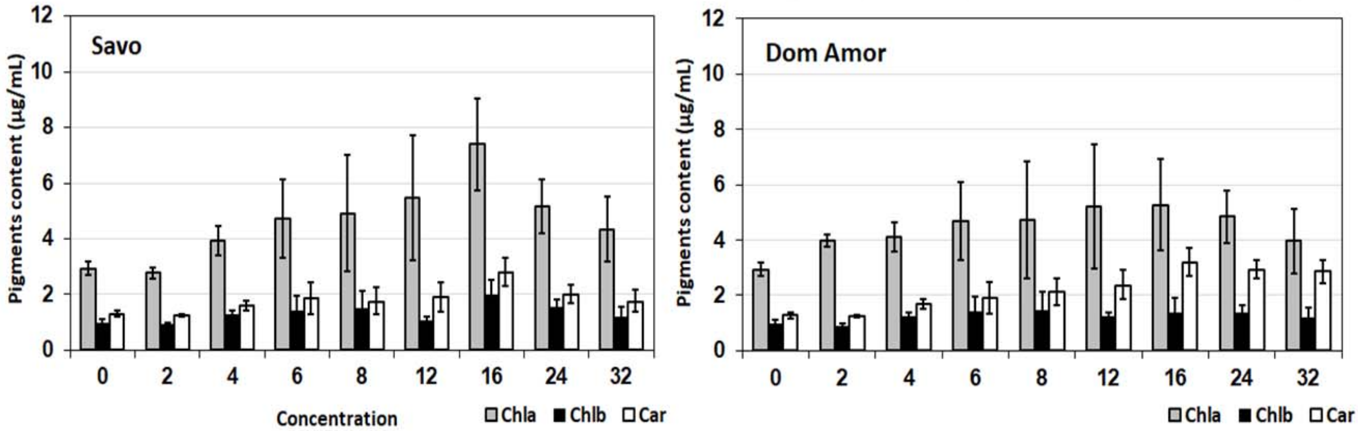


Fig. 2. Roots and shoots dry (DM) and fresh (FM) mass production in the presence of tested disinfectants after 10 days' cultivation introduced as standardized values of the reference control (water content in control = 1). Statistical significance: * P < 0.05; ** P < 0.01.

be toxic to a crop and restrict the agriculture mainly in saline regions. Many important cereals, vegetables and fruit crops are susceptible to Cl^- toxicity during cultivation (Xu *et al*. 2000), and this statement was confirmed also for the mustard crop during our study.

The last observed parameter during our experiments was the determination of photosynthetic pigments content. These results are presented on Fig. 3, and suggest a significant increase of Chla content in the presence of all disinfectants. Its production increased with the disinfectant's concentration, and maximal stimulation was confirmed during NaClO application when the Chla content in the NaClO concentration of 24 mL/L overextended its content in the control by 3.5 times. For this pigment stimulation, the following rank order may be arranged: NaClO > Savo > Dom Amor. However, Chla is the most abundant pigment in plant and absorbs red wavelengths of light; Chlb is not as abundant and probably evolved later. In presence of active chlorine, its concentration did not significantly change.

Cars are a class of accessory pigments and in plants; they contribute to the photosynthetic machinery and protect them against photo-damage. In the presence of all the tested disinfectants,



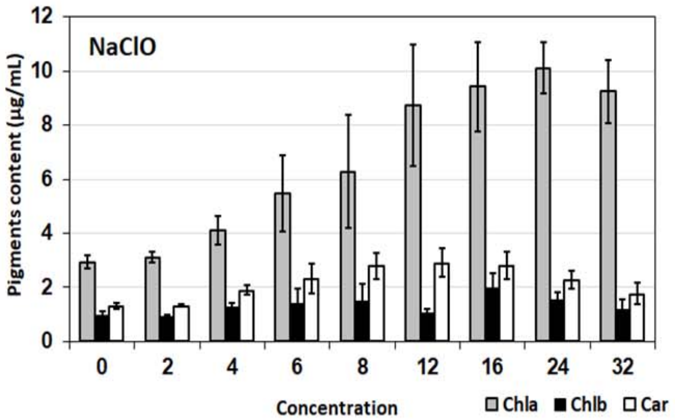


Fig. 3. Disinfectants effect on the production of chlorophyll a (Chla), chlorophyll b (Chlb) and carotenoids (Car) in the shoots of *S. alba* after 10 days' cultivation (µg/mL). Statistical significance was in all cases at least $P < 0.05$.

carotenoids concentration in the shoots were in higher applied concentrations significantly increased, however, did not overextend their content in the control by to 2.5 times for Dom Amor product. The stimulatory rank order for this case was: Dom Amor > NaClO > Savo. Chlorophyll content and fluorescence have been used to investigate the adverse effects of various environmental factors in experiments under controlled conditions. As presented by Khaleghi *et al.* (2012), drought is one of the factors affecting photosynthesis and chlorophyll content. The measurement of chlorophyll content and fluorescence has become established as a sensitive method for assessing the efficiency of PSII photosynthetically, as well as its role in environmental perturbations of photosynthesis. Some researchers have reported that chlorophyll content might estimate the influence of environmental stress on growth because these parameters were closely correlated with the rate of carbon exchange (Figueroa *et al.* 1997; Guo and Li 2000). As presented by Khaleghi *et al.* (2012), Kiani *et al.* (2008) and Guerfel *et al.* (2009), chlorophyll content (Chla, Chlb and total chlorophyll = Chla + Chlb) decreased under stress elicited by water toxicity. These statements can explain why the content of chlorophylls and carotenoids increased in the presence of free available chlorine in the concentrations used during our experiments with three disinfectant products.

Chlorine is classified as a plant micronutrient and is important for photosynthesis (Slabu *et al.* 2009; Marschner and Marschner 2012). In a high concentration, chlorine reduces plant growth, reduce yield (Albacete *et al.* 2008) and interfere with photosynthesis (Harjasuliha 1980). The critical concentration for chloride toxicity is estimated as 4 – 7 mg/L FAC for sensitive plants. In our tests, the FAC concentration which was used was 10-times lower, and adverse effects developed mainly in the roots. Shoots were more than two-times less sensitive than roots and their production of FM and DM increased. The water content in the shoots increased or was reduced only slightly, and this could explain why the photosynthetic pigment content also increased.

Conclusions

In low concentrations chlorine is classified as a plant micronutrient and the phytotoxic effects of its anion begin to appear only in higher concentrations. The obtained results confirmed that in *S. alba* crop roots are more sensitive to free active chlorine. All tested products – NaClO and the two commercial products Savo and Dom Amor, reduced roots growth more than dry (DM) and fresh mass (FM) production. DM and FM production correlate also with a strong reduction of water content (WC) in the roots. Slight shoots growth decreases as well, since DM and FM stimulation support photosynthetic pigments production, mainly that of chlorophyll a (Chla).

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References

Albacete A, Ghanem, ME, Martinez-Andujar C, Acosta M, Sanchez-Bravo J, Martinez V, Lutts S, Dodd IC, Perez-Alfocea F (2008) Hormonal changes in relation to biomass partitioning and shoot impairment in salinized tomato (*Solanum lycopersicum* L.) plants. J. Exp. Bot. 59: 4119-4131.

Amin MM, Hashemi H, Bovini AM, Hung YT (2013) A review on wastewater disinfection. Int. J. Environ. Health. Eng. 2: 22-30.

Añasco NC, Koyama J, Imai S, Nakamura K (2008) Toxicity of residual chlorines from hypochlorite-treated seawater to marine amphipod *Hyale barbicornis* and estuarine fish *Oryzias javanicus*. Water Air Soil Pollut. 195: 129-136.

Bush EA, Hong C, Stromberg EI (2003) Fluctuation of *Phytophthora* and *Phythium* spp. in components of a recycling irrigation system. Plant Dis. 87: 1500-1506.

Carrilo A, Puente ME, Bashan Y (1996) Application of diluted chlorine dioxide to radish and lettuce nurseries insignificantly reduced plant development. Ecotoxicol. Environ. Saf. 35: 57-66.

Cayanan CF, Dixon M, Zheng Y, Llewellyn J (2009) Response of container-grown nursery plants to chlorine used to disinfest irrigation water. Hort. Sci. 44: 164-167.

Drazic G, Mihailovic N (2005) Modification of cadmium toxicity in soybean seedlings by salicylic acid. Plant Sci. 168: 511-517.

Fargašová A (2016) Ecological risk assessment framework. Acta Environ. Univ. Comenianae (Bratislava) 24: 10-16.

Fargašová A (2017) A test battery approach for ecotoxicological evaluation of disinfectants prepared on the basis on sodium hypochlorite. Monatsh. Chem. 148, in press.

Fargašová A, Lištiaková J (2009) Cr and Ni simultaneous phytotoxicity and mutagenicity assay. Nova Biotechnol. Chim. 9: 107-112.

Figueroa ME, Fernández-Baco L, Luque T, Davy AJ (1997) Chlorophyll fluorescence, stress and survival in populations of Mediterranean grassland species. J. Vegetation Sci. 8: 881-888.

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. Ann. Rev. Plant Physiol. 31: 149-190.

Greenway H, Thomas DA (1965) Plant response to saline substrate. V. Chloride regulation in the individual organs of *Hordeum vulg*are during treatment with sodium chloride. Aust. J. Biol. Sci. 18: 505-524.

Guerfel M, Baccouri O, Boujnah D, Chaibi W, Zarrouk M (2009) Impact of water stress on gas exchange, water relations, chlorophyll content and leaf structure in the two main Tunisian olives (*Olea europaea* L.) cultivars. Sci. Hort. 119: 257-263.

Guo P, Li R (2000) Effects of light nocturnal temperature on photosynthetic organization in rice leaves. Acta Bot. Sinica 42: 13-18.

Hajrasuliha S (1980) Accumulation and toxicity of chloride in bean plants. Plant Soil 55: 133-138.

Hong CX, Moorman GW (2005) Plant pathogens in irrigation water: Challenges and opportunities. Crit. Rev. Plant Sci. 24: 189-208.

Khaleghi E, Arzani K, Moallemi N, Barzegar M. (2012) Evaluation of chlorophyll content and chlorophyll fluorescence parameters and relationships between chlorophyll a, b and chlorophyll content index under water stress in *Olea europea* cv. Dezful. Agric. Biosystems Engr. 6: 636-639.

Kiani SP, Maury P, Sarrafi A, Grieu P. (2008) QTL analysis of chlorophyll fluorescence parameters in sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions. Plant Sci. 175: 565-573.

Lichtenthaler HK, Wellburn AR (1983) Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 603: 591-592.

Marschner H, Marschner P (2012) Marschner's mineral nutrition of higher plants, 3rd ed. Elsevier/Academic Press, London; Waltham, MA, 672 p.

Mohammadi Z. (2008) Sodium hypochlorite in endodontics: an update review. Int. Dental. J. 58: 329-341.

Montero E, Cabot C, Poschenrieder C, Barcelo J (1998) Relative importance of osmotic-stress and ion specific effects on ABA-mediated inhibition of leaf expansion growth in *Phaseolus vulgaris*. Plant Cell Environ. 21: 54-62.

Nieuwenhuijsen MJ, Toledano MB, Eaton NE, Fawell J, Elliott P (2000) Chlorination disinfection byproducts in water and their association with adverse reproductive outcomes: a review. Occup. Environ. Med. 57: 73-85.

OECD 208: OECD guidelines for the testing of chemicals No. 208. (2006) Terrestrial plant test: Seedling emergence and seedling growth test. Organization for Economic Co-operation and Development, Paris, 19 p.

Parke J, Fisher P (2012) Treating irrigation water. Digger 56: 41-45.

Sapone A, Canistro D, Vivarelli F, Paolini M (2016) Perturbation of xenobiotic metabolism in *Dreissena polymorpha* model exposed *in situ* to surface water (Lake Trasimene) purified with various disinfectants. Chemosphere 144: 548-554.

Slabu C, Zörb C, Steffens D, Schubert S. (2009) Is salt stress of faba bean (*Vicia faba*) caused by Na^+ or Cl^- toxicity? J. Plant Nutr. Soil Sci. 172: 644-650.

Tavakkoli E, Rehgasamy P, McDonald GK (2010) High concentration of Na^+ and Cl^- ions in soil solution have simultaneous detrimental effects on growth of faba been under salinity stress. J. Exp. Bot. 61: 4449-4459.

Teakle NL, Tyerman SD (2010) Mechanisms of Cl^- transport contributing to salt tolerance. Plant Cell Environ. 33: 566-589.

Valica M, Hostin S. (2016) Electrochemical treatment of water contaminated with methylorange. Nova Biotechnol. Chim. 15: 55-64.

White PJ, Broadley MR (2001) Chloride in soils and its uptake and movement within the plant: a review. Ann. Bot. 88: 967-988.

Xu G, Magen H, Tarchitzky J, Kafkafi U (2000) Advances in chloride nutrition of plants. Adv. Agron. 68: 97-150.