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PHYTOTOXIC EFFECTS OF SELENIUM OXOACIDS AND SOME OF THEIR SALTS ON GROWTH OF *Brassica napus* L. SEEDLINGS

FITOTOKSYCZNY WPŁYW OKSOKWASÓW SELENOWYCH ORAZ ICH NIEKTÓRYCH SOLI NA WZROST SIEWEK *Brassica napus* L.

Summary: The aim of this study was to investigate the effects of selenium oxoacids and some of their salts of the type $MSeO_3$ and $MSeO_4$ (where X = Cd, Zn and Na_2) applied in the concentration range from 10^{-6} to 10^{-3} mol dm^{-3} on length of roots and shoots of rapeseed (*Brassica napus* L.) and to compare the toxic effect of selenates(IV) with that of corresponding selenates(VI). The set of Se-containing compounds was completed with H_2SeO_3 and an adduct of H_2SeO_4 with nicotinamide ($H_2SeO_4 \cdot$ nia) as well as with $Cd(SeCN)_2(nia)_2$. The studied compounds inhibited germination and growth of rapeseed seedlings. In general, root growth of rapeseed was inhibited to a greater extent than the shoot growth. The inhibitory effectiveness of the studied compounds related to root and shoot growth inhibition of *B. napus* plants was expressed by IC_{50} values. Se oxidation state affected the toxicity of the studied compounds. Compounds Na_2SeO_3 and H_2SeO_3 containing selenium as Se(IV) exhibited higher toxicity than Na_2SeO_4 and H_2SeO_4 with Se(VI). On the other hand, $CdSeO_4$ was more toxic than $CdSeO_3$. The phytotoxic effects of $CdSeO_4$, $ZnSeO_4$ and $Cd(SeCN)_2(nia)_2$ were compared with those of the corresponding sulphur analogues $CdSO_4$, $ZnSO_4$ and $Cd(SCN)_2(nia)_2$.

Keywords: rapeseed, germination, growth, cadmium, zinc, sodium, selenate(IV), selenate(VI)

Rapeseed (*Brassica napus* L.) is a very important agricultural and technical plant species and it represents also a staple for biofuel production [1]. Moreover, it could be also used for phytoremediation of metal-contaminated environment, mainly in removing cadmium and zinc from the soil [2, 3].

Besnard a Larher [4] found that cadmium treatment (150 μmol dm^{-3} $CdCl_2$) caused increased synthesis of phytochelatins in *B. napus* plants reaching 1.28 μmol SH/g fresh weight after 48 h treatment. Grispen et al. [5] exposed hydroponically grown 21-day-old seedlings of 77 *B. napus* accessions to 0.2 μmol dm^{-3} $CdSO_4$ for an additional 10 days

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and found that total Cd amount in shoots and shoot to root Cd concentration (S/R ratio or translocation factor) are independent parameters for Cd accumulation and translocation. In field experiments performed on metal contaminated soil these authors found significant correlations between Cd and Zn accumulation in shoots. Certain *B. napus* L. accessions were found to be suitable for phytoextraction of soils moderately contaminated with heavy metal.

Brennan and Bolland [6] investigated the relative effectiveness of soil-applied zinc and compared the yield and zinc content in canola plants grown in a greenhouse experiment using 2 alkaline soils from south-western Australia. The results showed that canola used effectively zinc fertilizer and found that the critical Zn concentrations in the youngest tissue, associated with 90 % of the relative yield, was 15 mg Zn kg^{-1}; corresponding value for dried shoots was 12 mg Zn kg^{-1}. Wenzel et al. [7] tested chelate-assisted phytoextraction of copper, lead and zinc using EDTA and canola plants (*Brassica napus* L., cv. Petranova). The plants were cultivated on a moderately polluted industrial soil (loamy sand) in the sub-continental climate of Eastern Austria. It was found that shoot biomass and water contents of canola were virtually unaffected by EDTA, revealing that canola can tolerate excessive metal concentrations in soil pore water. Metal concentrations in shoots increased considerably, but were insufficient to obtain reasonable extraction rates. Metal concentrations in roots decreased after each application of EDTA, possibly indicating metal removal from roots by free protonated EDTA, but increased again within several days.

Banuelos et al. [8] evaluated Se concentration in plant shoots of *Brassica napus* cv. Westar (canola) grown on seleniferous soil and found that leaf Se was as high as 470 mg Se kg^{-1} dry matter in canola and the reduction of total soil Se between preplant and the final harvest of canola plants was 47 %. Moreover, canola was found to be an excellent candidate for removing Se under the tested salinity conditions [9]. Ajwa et al. [10] studied Se uptake by canola plants grown in soils and found that tissues of canola accumulated much greater concentrations of Se from the inorganic SeO_4^{2-} treatment compared with the treatments with seleniferous organic materials since in soils amended with seleniferous organic materials, more than 80 % of the Se remained in soils after planting of canola. Banuelos et al. [11] found that canola irrigating with Se-laden effluent helps manage Se-laden effluent requiring treatment, and also produces economically viable Se-enriched crops.

The aim of this paper was to compare the inhibitory effects of selenium oxoacids and some of their salts, as well as two complex compounds $Cd(NCSe)_2(nia)_2$ and $Cd(NCS)_2(nia)_2$ on root and shoot growth of *B. napus* L.

Material and methods

For experiments following compounds were used: $CdSeO_3$, $CdSeO_4$, $ZnSeO_3$, $ZnSeO_4$, Na_2SeO_3, Na_2SeO_4, H_2SeO_3, H_2SeO_4.nia, $Cd(NCSe)_2(nia)_2$, $(CdSO_4)_3(H_2O)_8$, $ZnSO_4 \cdot 7H_2O$ and $Cd(NCS)_2(nia)_2$. These compounds were prepared according to Baudler [12] and Kráľová et al. [13, 14]. Analytical reagent-grade chemicals purchased from Lachema (Brno, Czech Republic) were employed for the preparation of all solutions. Freshly distilled water was used in all experiments.

The seeds of *Brassica napus* L. were placed in Petri dishes with a 14 cm diameter and filter paper on the bottom. In each Petri dish 58 seeds were evenly displayed on the surface of filter paper and the amount of solution used was 10 cm^3 per dish. Each concentration was duplicated. After 72 h exposure in the dark at mean air temperature (25 ± 0.5 ^oC) the length of roots and shoots of seedlings was measured. The applied concentration range of studied compounds was 10^{-5} to 10^{-6} mol dm^{-3}.

Results and discussion

Selenium oxoacids and some of their salts were found to inhibit root and shoot growth of some dicotyledonous species [15, 16]. In general, root growth of rapeseed seedlings was inhibited to a greater extent than the shoot growth (higher shoot growth inhibition induced H_2SeO_3 and Na_2SeO_3). From IC_{50} values related to inhibition of root and shoot growth by the studied compounds (Table 1) it is evident that the most effective inhibitor from the investigated set was $Cd(NCSe)_2(nia)_2$ (with IC_{50} values 77 μmol dm^{-3} for root and 134 μmol dm^{-3} for shoot growth inhibition). This complex is not ionic compound and it contains coordinated molecules of nicotinamide and $NCSe^-$, respectively [14]. Nicotinamide molecule is unidentate coordinated to Cd(II) atom through the ring nitrogen atom, $NCSe^-$ anionic ligands are also unidentate coordinated to Cd(II) atom and act as *N*-donor ligands. Comparable phytotoxic effect to that of $Cd(NCSe)_2(nia)_2$ exhibited $CdSeO_4$. Comparable root growth inhibition was observed with $CdSeO_4$ (IC_{50} = 78 μmol dm^{-3}).

The toxicity of studied compounds related to root growth inhibition decreased in the following order: $Cd(NCSe)_2(nia)_2$ ~ $CdSeO_4$ > $CdSeO_3$ > $ZnSeO_4$ > $H_2SeO_4 \cdot$ nia > Na_2SeO_3 > H_2SeO_3 > Na_2SeO_4 > $ZnSeO_3$ > $CdSO_4$ > $ZnSO_4$ > $Cd(NCS)_2(nia)_2$. For the toxicity of studied compounds related to shoot growth inhibition a similar rank was found: $Cd(NCSe)_2(nia)_2$ > Na_2SeO_3 > H_2SeO_3 > $H_2SeO_4 \cdot$ nia > $CdSeO_4$ > $ZnSeO_4$ ~ ~ $CdSeO_3$ > Na_2SeO_4 > $ZnSeO_3$ > $CdSO_4$ > $ZnSO_4$ > $Cd(NCS)_2(nia)_2$. While phytotoxicity of Na_2SeO_3, H_2SeO_3 and $ZnSeO_3$ was higher than that of Na_2SeO_4, H_2SeO_4 and $ZnSeO_4$, $CdSeO_4$ was more toxic than $CdSeO_3$.

Selenate(IV) is passively taken up into the plants and $CdSeO_3$ treatment resulted in relative low translocation of Cd into the shoots [17], probably due to insoluble Cd-Se complex formation in the roots. Consequently, the harmful effect of $CdSeO_3$ on the aboveground part of rapeseed seedlings was reduced. Selenate(VI) enters plant cells through a process of active transport mediated by sulfate transporters and $CdSeO_4$ treatment is connected with higher mobility of Cd within the plant and thus its toxicity is higher. Similar results to the above discussed ones were obtained previously by studying phytotoxic effects of the studied compounds on root and shoot growth of cress (*Lepidium sativum* L.) seedlings, however this species was much more sensitive to the treatment than *B. napus* what was reflected in lower IC_{50} values [15]. The corresponding IC_{50} values (in μmol dm^{-3}) related to inhibition of cress roots by the studied compound were as follows: 49.5 ($CdSO_4$), 20.2 ($CdSeO_4$), 52.9 ($CdSeO_3$), 8.1

Table 1

IC_{50} values related to inhibition of root and shoot growth of *B. napus* L. seedlings by the studied selenium oxoacids and some of their salts (C.L. – confidence limits)

Compound	$IC_{50} \pm C.L._{0.05}$ [mmol dm^{-3}]	
	root	shoot
$H_2SeO_4 \cdot$ nia	**0.162** (0.142–0.185)	**0.170** (0.103–0.299)
H_2SeO_3	**0.242** (0.230–0.255)	**0.165** (0.054–0.255)
$ZnSeO_4$	**0.123** (0.084–0.176)	**0.356** (0.204–0.709)
$ZnSeO_3$	**0.270** (0.189–0.534)	**0.710** (0.328–1.386)
Na_2SeO_4	**0.250** (0.136–0.324)	**0.433** (0.377–0.555)
Na_2SeO_3	**0.188** (0.126–0.255)	**0.141** (0.092–0.151)
$Cd(SeCN)_2(nia)_2$	**0.077** (0.056–0.105)	**0.134** (0.094–0.186)
$Cd(SCN)_2(nia)_2$	**1.292**	**1.031**
$CdSeO_4$	**0.078** (0.077–0.081)	**0.256** (0.177–0.404)
$CdSeO_3$	**0.119** (0.102–0.157)	**0.364** (0.262–0.555)
$CdSO_4$	**0.514** (0.459–0.544)	**0.940** (0.713–1.021)
$ZnSO_4$	**> 1.000**	**1.013**

($Cd(NCSe)_2(nia)_2$), 51.8 ($Cd(NCS)_2(nia)_2$), 22.6 ($ZnSeO_4$), 41.5 ($ZnSeO_3$), 63.7 ($H_2SeO_4 \cdot$ nia), 32.5 (H_2SeO_3), 91.0 (Na_2SeO_4) and 13.3 (Na_2SeO_3) and those related to cress shoot inhibition were 80.1 ($CdSO_4$), 43.5 ($CdSeO_4$), 76.9 ($CdSeO_3$), 19.5 ($Cd(NCSe)_2(nia)_2$), 323.7 ($Cd(NCS)_2(nia)_2$), 26.4 ($ZnSeO_4$), 61.5 ($ZnSeO_3$), 122.9 (H_2SeO_4.nia), 44.4 (H_2SeO_3), 130.0 (Na_2SeO_4) and 27.2 (Na_2SeO_3). Higher tolerance of *B. napus* plants to metal stress provide a possibility to utilize this industrial crop for remediation of heavy metal polluted soils.

Sulfur is one of the six macronutrients required by plants and is found in the amino acids cysteine and methionine and in a variety of metabolites. It is available to plants primarily in the form of anionic sulfate (SO_4^{2-}) present in soil and it is actively transferred into roots and then distributed, mostly unmetabolized, throughout the plant. Selenium (Se) has chemical properties similar to the sulfur, but slight differences can lead to altered tertiary structure and dysfunction of proteins and enzymes, if selenocysteine is incorporated into proteins in place of cysteine [18]. Selenocyanate, $SeCN^-$ is a highly toxic contaminant. Plants naturally detoxify SCN^- by converting it to dimethylsulfide and, by analogy with sulfur, it is possible that plants can also convert $SeCN^-$ to dimethylselenide. The first step of this pathway is the uptake of $SeCN^-$, which is most likely taken up actively by the plant (as are SeO_4^{2-} and selenomethionine) [19]. The higher toxicity of $Cd(NCSe)_2(nia)_2$ in comparison with $Cd(NCS)_2(nia)_2$ could also be connected with differences in stability constants of both compounds. The overall stability constant (β_2) of the complex compound $Cd(NCS)_2$ is 602.56 ($\beta_2 = 10^{2.78}$), whereas β_2 estimated for $Cd(NCSe)_2$ is only 199.53 ($\beta_2 = 10^{2.3}$) indicating three times lower stability of the compound with $NCSe^-$ ligands [20]. Due to release of $NCSe^-$ anion from the complex Cd could interact with suitable target groups on biomolecules. Moreover, $NCSe^-$ anion may exert its harmful effect, too.

Summarizing it could be concluded that rapeseed belongs to plants which are tolerant to metal treatment. It is able to accumulate substantial amounts of metals in different plant organs, including seeds. Besides of potential utilization of *B. napus* plants for phytoremediation of soils contaminated with toxic metals, from seeds of *B. napus* phytofortificated with essential metals (*eg* Zn or Se) rapeseed press cakes can be prepared which could be used as excellent nutritive animal fodder.

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FITOTOKSYCZNY WPŁYW OKSOKWASÓW SELENOWYCH ORAZ ICH NIEKTÓRYCH SOLI NA WZROST SIEWEK *Brassica napus* L.

S t r e s z c z e n i e

Zbadano wpływ oksokwasów selenowych i kilku soli typu $MSeO_3$ i $MSeO_4$ (gdzie X = Cd, Zn i Na_2) stosowanych w stężeniach od 10^{-6} do 10^{-3} mol · dm^{-3} na długość korzeni i kiełkowanie nasion rzepaku (*Brassica napus* L.) i porównano skutki toksycznego działania selenianów(IV) oraz selenianów(VI). Związki zawierające Se zostały uzupełnione o H_2SeO_3 i związek addycyjny H_2SeO_4 z amidem nikotynowym (H_2SeO_4 · nia), jak również o $Cd(SeCN)_2(nia)_2$. Analizowane związki hamowały kiełkowanie i wzrost rzepaku. Wzrost korzenia nasienia rzepakowego był hamowany w większym stopniu niż wzrost pędu. Inhibicyjne działanie analizowanych związków na ukorzenianie się i wzrost roślin *B. napus* było wyrażony przez wartości IC50. Stopień utlenienia selenu miał wpływ na toksyczność analizowanych związków. Związki Na_2SeO_3 i H_2SeO_3 zawierające selen Se(IV) były bardziej toksyczne niż Na_2SeO_4 i H_2SeO_4 z selenem Se(VI). Z drugiej strony, $CdSeO_4$ był bardziej toksyczny niż $CdSeO_3$. Fitotoksyczne działania $CdSeO_4$, $ZnSeO_4$ i $Cd(SeCN)_2(nia)_2$ były porównywalne ze skutkami wywołanymi przez odpowiednie analogi siarczanowe: $CdSO_4$, $ZnSO_4$ i $Cd(SCN)_2(nia)_2$.

Słowa kluczowe: rzepak, kiełkowanie, wzrost, kadm, cynk, sód, selenian(IV), selenian(VI)