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Role of sulphate (SO_4^{2-}) in improvement the growth of rocket plants (*Eruca sativa* L.) under selenate (SeO_4^{2-}) levels

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Abstract

Selenium is an essential microelement for plant growth and productivity, but high selenium levels impose toxicity in plants. A pot experiment was conducted to investigate the role of sulphate concentrations for ameliorate the adverse effects of high selenate levels on growth of 30-day old rocket plants (cv Egyptian baladi). High Se levels preferentially decreased the biomasses, succulent values in leaves and roots as well as leaf area of rocket plants. This was associated with a suppression of photosynthetic pigments and foliar nutrient elements (K, P, S, Mg and N as NO_3^-) with an increase of Se content. Supplementation the high Se-containing medium with double SO_4^{2-} concentration in 1/10 Hoagland solution markedly antagonized Se uptake and its assimilation reflecting the shift- off to some extent- the inhibitory effect of high Se on growth through induction of minerals uptake and biosynthesis of photosynthetic pigments. In addition; changing the protein profile revealed variations in protein patterns and a decrease in selenoprotein biosynthesis by increasing sulphate concentration. However, supplementation of plants with low selenate (2 ppm) level combined with high sulphate (2 ppm) concentration resulted in an optimal growth.

Key words: biomass -sulphate transporter - nutrient imbalance –nitrate –antagonistic effect-selenoprotein.
Abbreviations: Carot_Carotenoids; Chl *a*, Chl *b*_Chlorophyll *a*, *b;* DM_Dry biomass; FM)_Fresh biomass; SeCys_selenocysteine; SeMet_selenomethionine.

Introduction

Selenium has been considered an essential microelement for humans, animals and some species of microorganisms (Terry et al*.,* 2000). Selenium exists in soil in several inorganic forms as elementary selenium (Se^0), selenide (Se^{2-}), selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) as well as in organic forms such as selenomethionine (SeMet) and selenocysteine (SeCys). The bioavailability of Se is determined by its chemical form, the soil redox potential, pH and clay content (Pyrzynska, 2009). Under oxidizing alkaline soil conditions, selenite is strongly adsorbed on iron oxide surfaces and becomes less available than selenate for plant growth (Neal, 1995). Selenate chemically analogous to sulphate ion, is actively transported into roots *via* sulphate transporters in plasma membrane and transported into leaves and chloroplast where selenate is reduced to selenite, then selenide *via* S-assimilation pathway which is enzymatically incorporated into SeCys and SeMet (Terry et al., 2000; Sors et al., 2005). Selenium was shown to affect several physiological and biochemical processes in different plant species and hence on plant growth and productivity. Se often exerts a dual effect on plant growth, whereas low Se concentrations can stimulate the growth of plants and counteract many types of environmental stresses. On the other hand, higher Se levels resulted in several toxic effects include stunted root growth, reduced biomass, chlorosis, reduced photosynthetic efficiency, disturbance of nutrient imbalance and ultimately plant death (Hasanuzzaman et al., 2010; Nancy and Arulselvi, 2014). Sulphur is a macronutrient element that essential for plant growth and it is absorbed as inorganic form *via* several SO_4^{2-} transporters and permeases. Then it might be either accumulated in vacuoles or it is reduced by sulphurylase and incorporated to various organic compounds and S-containing proteins (Terry et al., 2000; Mukwevho et al., 2014). It has been reported that due to chemical similarity between SO_4^{2-} and SeO_4^{2-}, an antagonistic phenomena for transporters and reductant agents during their uptake and assimilation is clearly recorded depending upon the concentration of both anions (Freeman et al., 2010). The objective of this study is to evaluate the role of increasing SO_4^{2-} level in the mitigation of high selenium level toxicity on growth parameters, chlorophyll pools, elements uptake and protein profile of 30-d old rocket plants.

Results and Discussion

Changes in growth parameters of garden rocket plants under selenate toxicity

Supplementation the nutrient medium (S-poor or S-enriched) of rocket plants with 2 ppm Se significantly increased FM, DM and leaf area, compared to those in absence of Se. On the other hand, in presence of high Se level (10 ppm), there was a significant suppression of biomass in leaves and roots (Table 1). Moreover, the decrease in S-poor medium was markedly greater than those in S-enriched medium. The reduction in FM of leaves and roots in S-poor medium contaminated with 10 ppm was about 78% and 74 % respectively compared to those in absence of Se. The corresponding values in S-enriched medium were 34 % and 47 % respectively.

In parallel, increasing Se concentration resulted in a significant decrease of succulence (FM/DM) in leaves and roots (Fig. 1), however, the succulence of leaves and roots in presence of higher S concentration were greater than those grown in poor S. These findings are in agreement with several studies indicated the dual effects of Se as growth-promoting or growth-inhibitor factor (Põldma et al., 2013; Nancy and Arulselvi, 2014). In addition, increasing SO_4^{2-} concentration in high Se-contaminated nutrient medium diminishes the inhibitory effect of Se on the growth of rocket plants. These observations indicate that increasing SO_4^{2-} levels might decrease Se uptake and its assimilation.

Changes in foliar selenate accumulation of garden rocket plants under selenate toxicity

There was a significant increase of total Se content in garden rocket leaves in response to increasing Se concentration in the S-poor or S-enriched nutrient medium, however, the foliar content in the latter was significantly less than of the former (Table 2). The total Se content at 10 ppm Se-treated plants in presence of high S level was 44% compared to those in S-poor. Moreover, the increase of total Se was related, mainly to marked increase of organic-Se fraction. Both of Se-deficiency and Se-toxicity triggers serious problems for human health. So; the determination of Se content in rocket plants is a crucial where plants represent the major Se dietary source for human. Malagoli et al. (2015) indicated that the appropriate Se content in human diet must be in range 50 to 55 µg/day.

In this study, increasing of suphate level in Se-contaminated nutrient media resulted in a marked decrease of foliar total and organic-Se and that associated with an increase of foliar S content. White et al. (2004) and Freeman et al. (2010**)** reported that the enhancement of both shoot growth and S content of *Arabidopsis thialana* and *Stanleya pinnata* respectively, was related to an increase of SO_4^{2-} to SeO_4^{2-} ratio in the nutrient medium. Also, Hajiboland and Amjad (2007) showed that increasing of Se at low S level exerted an inhibitory effect on biomass of cabbage, kohlrabi and alfalfa plants. Pilon-Smits et al. (1999) concluded that $SO_4^{2-}$and SeO_4^{2-} are taken up *via* SO_4^{2-}- permease and then reduced by ATP-sulphurylase in presence of NADPH.H as reductants. Furthermore, Tamaoki et al. (2008) and Feng et al. (2013) have reported that SO_4^{2-} caused antagonistic effect on SeO_4^{2-} uptake due to competition for SO_4^{2-}-transporter on plasma membrane and reductants. Therefore, the improvement of rocket plants growth, under this study could be attributed to the competition between SO_4^{2-} and SeO_4^{2-} on the same carriers during transportation and the reducing agents, depending on their ratios in the nutrient medium. At high SeO_4^{2-}/SO_4^{2-} ratio (S-poor medium), there was a marked increase of SeO_4^{2-} uptake in expense of SO_4^{2-} resulting in an increase of Se accumulation and inhibition of rocket plants growth and *vice versa* at high SO_4^{2-}/ SeO_4^{2-} ratio.

Terry et al. (2000) and Hajiboland and Amjad (2007) concluded that substitution of Se to S as seleno-amino acids (SeCyst or SeMet) instead of cysteine and methionine might resulted in disturbance in protein biosynthesis and tertiary structure of some enzymes. Thus, the decrease of foliar organic –Se of rocket plants at high SO_4^{2-}/ SeO_4^{2-} ratio, compared to those at low SO_4^{2-}/SeO_4^{2-} ratio might indicate a decrease of incorporation of seleno-amino acids in specific proteins of several enzymes such as permeases associated with plasma membranes resulting in a suppression of inhibitory effect of Se on K, P, S, Mg and N (as NO_3^-) transportation and hence stimulate the metabolic processes and finally increase in the biomass of rocket plants.

Changes in photosynthetic pigments of garden rocket leaves under selenate toxicity

The total photosynthetic pigments content in leaves of 30-d old of highly Se-treated plants was significantly decreased compared to those in absence of Se (Table 3). The suppression of total photosynthetic pigments was mainly related to decrease of Chl *a* and Chl *b* contents. The decrease in Chl *a* in highly Se levels with S-poor and S-enriched media was 51% and 36 % respectively compared to those in absence of Se. The corresponding values for Chl *b* were 70% and 63% respectively (Table 3).

There was a significant increase of Carot and Carot to Chl (*a* + *b*) ratio in presence of high SeO_4^{2-} level. Addition of double S level to the nutrient medium resulted to a marked decreases of these parameters comparing to S-poor plants (Table 3, Fig. 2). These results might reveal that increasing S level in the nutrient medium could shift off -to some extent- the inhibitory effect of Se on the photosynthetic machinery, and therefore, improve the growth of rocket plants. In addition, increase of Carot content and Carot/Chl *a* + *b* ratio might indicate the role of Carot as a defense mechanism against photooxidation (Ismail et al., 2017).

Changes in foliar nitrate content of garden rocket plants under selenate toxicity

Data in Table 2 clearly demonstrated that addition of low SeO_4^{2-} levels to nutrient media insignificantly changed the foliar NO_3^- content of rocket plants. On the other hand, supplementation the nutrient medium with high Se level resulted in a significant decrease of foliar NO_3^- content, compared to those in absence of Se (Table 2). The decline of foliar NO_3^- was reached to 61% and 56 % in S-poor and S-enriched media, respectively. In addition, it is noted that increasing in SO_4^{2-} level in the nutrient medium (S-enriched) resulted in a significant decrease of foliar NO_3^- compared to S-poor medium; the decrease reached up to 23% (Table 2).

It is shown that foliar NO_3^- content was significantly decreased under either presence or absence of Se with S-enriched medium compared of those under S-poor medium. These findings might be related to the competition among NO_3^-, SO_4^{2-} and SeO_4^{2-} on ATP- associated plasma membrane protein transporters and sources of H-donor. Guerrero et al. (1981) stated that NO_3^- absorption is ATP-dependent and assimilated *via* independent $NADP(H)^+$ nitrate reductase. Similarly, SO_4^{2-} and SeO_4^{2-} are absorbed by activated SO_4^{2-}

Table 1. The effect of selenate and elevated sulphate on fresh and dry biomasses (g. plant^{-1}) in the leaves and roots and leaf area of 30 d-old garden rocket plants.

Treatment (ppm)	Leaves		Roots		Leaf area
	FM	DM	FM	DM	(cm^2)
1 SO_4^{2-}/0 SeO_4^{2-}	5.14±0.41^a	0.59±0.07^b	1.21±0.08^d	0.14±0.02^c	5.80±0.44^c
1 SO_4^{2-}/2 SeO_4^{2-}	8.01±0.67^b	0.99±0.10^d	1.57±0.12^c	0.18±0.02^a	8.33±0.79^d
1SO_4^{2-}/10 SeO_4^{2-}	1.15±0.08^d	0.21±0.02^a	0.32±0.02^a	0.06±0.01^d	3.72± 0.43^a
2 SO_4^{2-}/0 SeO_4^{2-}	6.78±0.73^f	0.74±0.08^c	1.50±0.12^c	0.16±0.02^{ac}	7.30±0.78^g
2 SO_4^{2-}/2 SeO_4^{2-}	9.75±1.02^c	1.06±0.09^d	2.44±0.19^f	0.25±0.03^b	9.86±1.01^b
2 SO_4^{2-}/10 SeO_4^{2-}	4.46±0.36^a	0.68±0.07^c	0.79±0.06^g	0.11±0.01^c	4.84±0.37^n

Values are the means ± SE (*n* = 5). Means indexed by the same superscript letter in the column are not significantly different at P ≤ 0.05 (LSD test).

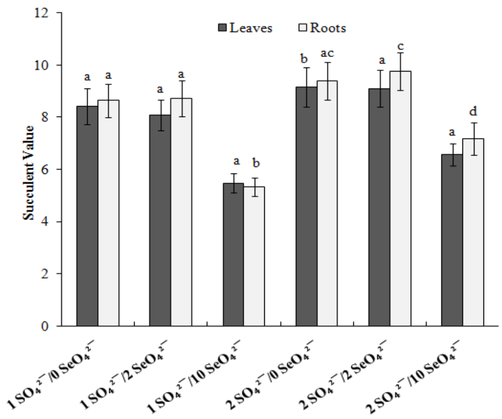

Fig 1. The effect of selenate and elevated sulphate on succulent value of the leaves and roots of 30 d-old garden rocket plants. Values are the means ± SE (*n* = 5). Different letters for each group of bars indicate the significant differences (*P* ≤ 0.05, LSD test).

Table 2. The effect of selenate and elevated sulphate on foliar selenium and nitrate contents of 30 d-old garden rocket plants.

Treatment (ppm)	Inorganic Se	Organic Se	Total Se	NO_3^-
		(µmol Se . g^{-1} DM)		(µmol. g^{-1} DM)
1 SO_4^{2-}/0 SeO_4^{2-}	00.00±00.00^b	0.00±0.00^c	0.00±0.00^a	39.24±2.92^b
1 SO_4^{2-}/2 SeO_4^{2-}	33.82± 2.56^c	52.36±4.52^a	86.18±4.53^b	37.98±3.18^b
1 SO_4^{2-}/10 SeO_4^{2-}	129.41±9.87^a	792.65±49.51^d	922.06±45.80^d	11.39±0.91^f
2 SO_4^{2-}/0 SeO_4^{2-}	0.00±0.00^b	0.00±0.00^c	0.00±0.00^a	30.35±1.97^a
2 SO_4^{2-}/2 SeO_4^{2-}	42.94±5.10^f	21.47±1.92^b	64.41±5.16^b	25.29±2.08^a
2 SO_4^{2-}/10 SeO_4^{2-}	115.29±10.76^d	292.36±23.12^f	407.65±29.03^f	17.22±1.56^c

Values are the means ± SE (*n* = 3).Means indexed by the same superscript letter in the column are not significantly different at P ≤ 0.05 (LSD test).

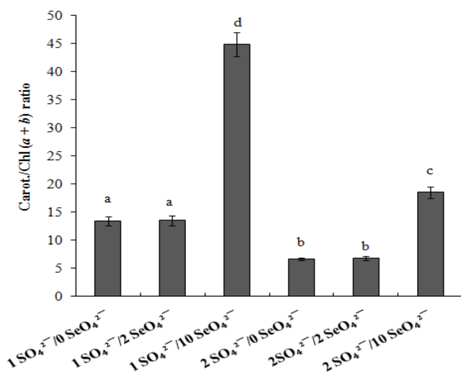

Fig 2. The effect of selenate and elevated sulphate on the Carot./Chl (*a* + *b*) ratio in the leaves of 30 d-old garden rocket plants. Values are the means ± SE (*n* = 3). Different letters indicate the significant differences (*P* ≤ 0.05, LSD test).

Table 3. The effect of selenate and elevated sulphate on photosynthetic pigments content in the leaves of 30d-old garden rocket plants. Values are the means of 3 independent replicates ± SE.

Treatments (ppm)	Pigment fractions ($mg.g^{-1}$ FM)			
	Chl. *a*	Chl. *b*	Carot.	Total pigments
1 SO_4^{2-}/0 SeO_4^{2-}	9.02±0.70^{d}	5.36±0.44^{c}	1.93±0.20^{a}	16.31±1.03^{c}
1 SO_4^{2-}/2 SeO_4^{2-}	9.35±0.85^{d}	5.08±0.49^{c}	1.95±0.12^{a}	16.38±1.18^{c}
1 SO_4^{2-}/10 SeO_4^{2-}	4.40± 0.32^{a}	1.62±0.09^{f}	2.67±0.29^{b}	8.69±0.74^{a}
2 SO_4^{2-}/0 SeO_4^{2-}	12.91±1.06^{c}	6.60±0.84^{a}	1.26±0.15^{c}	20.77±2.48^{b}
2 SO_4^{2-}/2 SeO_4^{2-}	11.75±0.98^{c}	6.24±0.65^{a}	1.22±0.08^{c}	19.21±2.16^{bc}
2 SO_4^{2-}/10 SeO_4^{2-}	8.31±0.71^{d}	2.42±0.19^{d}	1.98±0.21^{a}	12.71±1.09^{d}

Values are the means ± SE (*n* = 3).Means indexed by the same superscript letter in the column are not significantly different at P ≤ 0.05 (LSD test).

Table 4. The effect of selenate and elevated sulphate on the nutrient elements content in the leaves of 30 d-old garden rocket plants.

% Nutrient elements	Treatments (ppm)					
	1 SO_4^{2-}/0 SeO_4^{2-}	1 SO_4^{2-}/2 SeO_4^{2-}	1 SO_4^{2-}/10 SeO_4^{2-}	2 SO_4^{2-}/0 SeO_4^{2-}	2 SO_4^{2-}/2 SeO_4^{2-}	2 SO_4^{2-}/10 SeO_4^{2-}
K	46.20	45.05	31.45	44.41	45.03	40.52
Ca	27.08	31.20	49.09	22.82	24.10	33.29
S	14.94	13.29	6.71	22.97	21.51	11.82
P	5.95	4.58	2.30	4.04	3.68	4.46
Mg	0.66	0.81	0.83	0.89	1.08	1.50
Cu	2.01	1.55	0.68	1.84	1.59	1.47
Zn	0.92	1.20	0.76	1.71	1.48	1.69
Cl	1.73	2.44	1.83	1.08	0.95	1.94
Se	0.00	0.13	6.13	0.00	0.17	3.38
Total %			100			

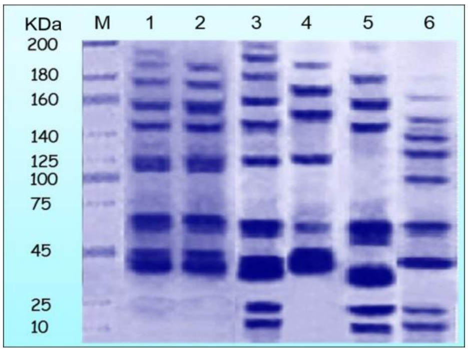

Fig 3. *SDS-PAGE analysis of foliar protein patterns of 30 d-old garden rocket plants supplemented with different concentrations of selenate in S-poor and S-enriched nutrient medium. Lane M: molecular mass (KDa) markers; Lanes from 1 to 6 were control (1 SO_4^{2-}/0 SeO_4^{2-}), (1 SO_4^{2-}/2 SeO_4^{2-}), (1 SO_4^{2-}/10 SeO_4^{2-}), (2 SO_4^{2-}/0 SeO_4^{2-}), (2 SO_4^{2-}/2 SeO_4^{2-}), (2 SO_4^{2-}/10 SeO_4^{2-}) treatments, respectively.*

transporters and assimilated by ATP-sulphurylase in presence of NADP(H)$^+$ (Terry et al*.,* 2000; Sors et al., 2005).

Changes in foliar nutrient elements contents of garden rocket plants under selenate toxicity

Addition of high Se concentration to S-poor nutrient medium markedly decreased the foliar macronutrients K, S and P, whereas increased Ca and Se contents. The content (as %) of K, S and P decreased from 46.20, 14.94 and 5.95 in control (1 SO_4^{2-}/0 SeO_4^{2-}) to 31.45, 6.71 and 2.30 respectively at high Se level (1 SO_4^{2-}/10 SeO_4^{2-}), while, Ca content increased from 27.08 to 49.09 (Table 4). Conversely, increasing S level in high Se-contaminated nutrient medium resulted in a marked decrease of Ca and Se accumulation with increasing of K, P, Mg, S, Cu and Zn contents, compared to those grown in S-poor and high Se nutrient medium (Table 4). During this study, increasing SO_4^{2-} level in Se-enriched medium resulted in a markedly increase of foliar K, S, P, Mg, Cu, N as NO_3^- and decrease of Se contents compared to those of S-poor medium. These findings were associated with an enhancement of Chl *a* and *b* as well as growth of rocket plants. Thus, the enhancement of growth at high SO_4^{2-}/SeO_4^{2-} ratio, could be related to suppress ROS generation and peroxidation of plasma membranes (Vikhreva et al**.**, 2002) resulting in improvement of water and nutrients uptake and allocation. In accordance with these results, many authors (White et al., 2004; Germ et al., 2007; Ahmed, 2010) have reported that increasing S concentrations in Se containing medium greatly increased the accumulation of K, P, N, Zn and S in various plant genera and hence improved the growth.

Garifullina et al. (2003) reported that Se can substitute for S in Fe-S cluster of chloroplasts resulting in disturbance of

Table 5. Survey of foliar protein bands in the 30 d-old garden rocket plants among the interaction between selenate and sulphate in the nutrient medium (A, 1 SO_4^{2-}/0 SeO_4^{2-}; B, 1 SO_4^{2-}/2 SeO_4^{2-}; C, 1 SO_4^{2-}/10 SeO_4^{2-}; D, 2 SO_4^{2-}/0 SeO_4^{2-}; E, 2 SO_4^{2-}/2 SeO_4^{2-}; F, 2 SO_4^{2-}/10 SeO_4^{2-}).

M.M	Treatments (ppm)					
M.M	A	B	C	D	E	F
195	+	+	+	+	+	+
189	+	+	+	+	+	+
178	+	+	+	+	+	+
170	-	-	+	-	-	-
166	-	-	-	+	-	-
157	+	+	+	-	+	+
150	+	-	-	-	-	-
146	-	-	+	-	-	+
142	+	+	+	+	+	+
127	-	-	+	-	-	+
121	+	+	+	+	+	+
70	+	+	-	-	-	-
66	+	+	+	+	+	+
62	-	-	+	-	-	-
51	-	-	-	-	+	-
41	+	+	-	-	-	-
27	-	+	-	-	-	-
21	+	+	-	+	-	-
17	-	-	+	-	+	+
12	-	-	+	-	+	+
No. of bands	11	11	13	8	10	11

Where: + = band present, - = band absent

Table 6. Pearson correlation coefficients (r) between total Se content and each of dry mass of leaves and roots, leaf area, total pigments and S content.

	Total Se	Leaf DM	Root DM	Leaf area	Total pigments
Leaf DM	-0.752**				
Root DM	-0.736**	0.888**			
Leaf area	-0.723**	0.881**	0.925**		
Total pigments	-0.866**	0.684**	0.709**	0.696**	
S content	-0.799**	0.663**	0.753**	0.741**	0.936**

** Correlation is significant at the 0.01 level (2-tailed).* Correlation is significant at the 0.05 level (2-tailed).

electron transport and generation of ROS, hence inhibiting the photosynthesis. In addition, Stibilj et al. (2011) concluded that the decrease of K, Fe, Mn and Zn in Se-exposed chicory leaves might result in disturbance in photosynthesis and biochemical processes, and hence suppressed the growth. Thus, the improvement in growth of rocket plants at S-enriched medium with high Se levels could be explained as a result of increasing foliar macronutrients (N, S, P, K and Mg) which leading to increase of chlorophylls biosynthesis and induction of photosynthetic machinery.

It is clearly shown that increasing of Se levels in both S-containing media of rocket plants resulted in a marked increase of foliar Ca content. Similarly, Khattab (2004) and Hawrylak-Nowak (2008) have reported that increasing Se levels in feeding media of rocket and Zea plants, respectively increased Ca uptake and its accumulation. While, Hyun et al. (2006) reported that SO_4^{2-} depressed Se uptake but enhanced Ca absorption. Toler et al. (2007) concluded that Ca plays an important role in maintenance of cellular membranes integrity. Thus, the increase in foliar Ca content of rocket plants grown in SO_4^{2-}/SeO_4^{2-} media might be attributed to increase of Ca^{2+} transportation in its role in protection of plasma membrane integrity resulting in an improvement the growth of rocket plants.

Changes in protein profile of leaves of garden rocket plants under selenate toxicity

Results in Table 5 and Fig. 3 showed that eleven distinct protein bands are found in control leaves with M.M. ranged from 195 KDa to 21 KDa (Lane 1). Supplementation the S-poor nutrient medium with 2 or 10 ppm Se resulted in a great variation of soluble protein bands in treated leaves compared to control. Under application of 2 ppm Se one polypeptide band with M.M. of 27 KDa was markedly appeared (Lane 2). On the other hand, treatment with 10 ppm Se resulted in an appearance of six new protein bands with M.M. of 170, 146, 127, 62, 17 and 12 KDa, accompanied with disappearance of 150, 70, 41 and 21 KDa bands (Lane 3). Increasing S concentration of the nutrient medium (S-enriched medium) leads to appearing of one polypeptide band with M.M. of 166 KDa with disappearance of 157, 70 and 41 KDa bands compared to those of S-poor medium (Lane 4). Three polypeptides with M.M. of 51, 17 and 12 KDa were markedly appeared with disappearance of 150, 70, 41 and 21 KDa (Lane 5) in the enriched-S and 2 ppm Se-treated leaves. While, four polypeptides with M.M. of 146, 127, 17 and 12 KDa were appeared in the enriched-S and 10 ppm Se-treated leaves (Lane 6) with disappearance of 150, 70, 41 and 21 KDa bands.
Perez-Alfocea et al. (1996) reported that changes of protein pattern are accompanied by several biological processes

which protect an organism from various stresses. In this study, subjection of rocket plants to Se-containing media resulted in appearance of polypeptide bands with M.M of 170, 146, 127, 62, 51, 27, 17 and 12 KDa indicating the synthesis of special selenoproteins. Cheng et al. (2006) concluded that incorporation of Se in some enzymes protein could increase the electrophilic properties and increasing their activities. Whereas, Terry et al. (2000) and Hajiboland and Amjad (2007) reported that biosynthesis of selenoproteins might result in an inhibition of several enzyme activities. Thus appearance of one selenoprotein band (27 KDa) in low Se level (Lane 2) might be considered as an inducer factor for growth (Fig. 3), while appearance of the bands with M.M of 170, 146, 127, 62, 17 and 12 KDa at high Se level might be as an inhibitor for the growth. In addition, the diminished the selenoprotein bands to four only at high SO_4^{2-} level (Lane 6) compared to six bands at low SO_4^{2-} (Lane 3) might indicate the decrease of the inhibitory effect of synthesized selenoproteins and hence improved the growth of rocket plants.

Correlation analyses

Results in Table 6 showed that total selenium content of 30 d-old rocket plants was highly negative correlated (p = 0.01) with leaf and root dry masses (- 0.752, - 0.736), leaf area (- 0.723), total pigments(- 0.853), sulfur content (- 0.799). Highest positive correlation (p = 0.01) was revealed between sulfur content and leaf and root dry masses, leaf area and total pigments content (0.663, 0.753, 0.741, 0.936).

Materials and methods

Plant materials

Garden rocket (*Eruca sativa* L. cv. Egyptian baladi) seeds were surface sterilized by soaking in 4 % (v/v) sodium hypochlorite, then washed several times with tap water followed by distilled water and finally soaked in aerated distilled water for 24 h.

Growth conditions

The soaked seeds were transferred into plastic pots filled with acid-washed quartz sand and placed under natural environmental conditions (photoperiod 16/8 h light/dark, 12/23±2 ˚C; PPFD, 23 μmol m^{-2} sec^{-1}). The pots were divided into two sets; I, the pots were irrigated with 1/10 strength Hoagland solution (Epstein, 1972) contained 1 ppm sulphur (S-poor nutrient) and supplemented with either 0, 2 or 10 ppm Se; II, the pots were irrigated with 1/10 strength Hoagland solution contained 2 ppm sulphur (S-enriched nutrient) and supplemented with either 0, 2 or 10 ppm Se. Sulphur was supplemented as $MgSO_4$ and selenium as sodium selenate. All treatments are in triplicates. The irrigation was carried out every two days interval. At 30 d-old (from sowing), homologous plants were selected, washed to remove adhering sand, dissected to leaves and roots and saved for chemical analyses. Other samples were dried to constant weight for estimation dry mass and Se content. Most analyses were performed on the leaves using the full expanded youngest two leaves.

Growth parameters

Homologous plants (three replicates) were removed, washed, gently blotted, dissected to roots and leaves then weighed separately for roots and leaves fresh biomass (FM.) determination. The dry biomass (DM) was determined after drying the samples in an oven at 60 $^{\circ}$C till constant weight. Total leaf area (normal foliage leaves) was measured by digital planimeter (Placom KP-90) to the nearest cm². The succulent values were estimated as FM/DM.

Chemical analysis

Determination of total and inorganic-Se content

Extraction**:** According to Krishnaiah et al. (2003), ten ml of 1:1 (v/v) mixture of sulphoric acid and nitric acid were added to an aliquot of dry mass (5 g) of leaves and heated until the mixture becames clear, cooled, filtrated and make to appropriate volume. For extraction of inorganic-Se, similar previous procedure was carried out but 10 ml of 15 % HCl was added instead of H_2SO_4 : HNO_3.
Determination**:** The procedure of Krishnaiah et al. (2003) was carried out in which 5 ml of concentrated HCl and 2 ml of 1.5 % of a mixture of 2, 4-dinitrophenyl hydrazine hydrochloride (2,4-DNPH) and *N*(1-naphthyl) ethylenediamine dihydrochloride (NEDA) reagent were added to 1 ml of total- or inorganic-Se extract, stand for 10 min with shaking, diluted to standard volume and then the colour was measured at 520 nm against a calibration curve as Na-selenate.

Determination of photosynthetic pigments content

The photosynthetic pigments chlorophyll *a*, *b* (chl *a*, chl *b*) and carotenoids (Carot) were determined according to the method described by Inskeep and Bloom (1985). The Carot content was calculated according to Lichtenthaler (1987).

Determination of nutrient elements

According to the method of Taspinar et al. (2009); aliquots of DM were transferred to wavelength dispersive X-ray-Analysis Model: (Oxford) attached to a scanning electron microscope (WDXRSM, JEOL, JSM-5300). This instrument was controlled by a software computer for determination of K, Ca, S, P, Mg, Cu, Zn, Cl and Se contents. The measurements were calculated as a percent to each other.

Estimation of nitrate content

Nitrate was estimated according to the method of Johnson and Ulrich (1950).

Protein electrophoresis

Sodium dodecyl sulphate (SDS-PAGE) gel electrophoresis procedure was carried out according to Laemmli (1970).

Statistical analyses

Statistical analyses were done using (SPSS/version 22) software. The data were subjected to one-way ANOVA for variance test, least significant difference (LSD) at $p \leq 0.05$. All treatments were replicated three times except growth parameters were five replicates and results are given as mean ± standard errors (SE). Pearson correlation coefficient test was conducted to evaluate the relation between total selenate content, leaf and root fresh and dry masses, leaf area, total pigments content, sulfur content.

Conclusion

Low Se level stimulated the growth of rocket plants in presence of either S-poor or S-enriched nutrient media, however high Se level suppressed the growth of plants. Application of high SO_4^{2-} levels markedly prevents the inhibitory effect of high Se. This might be attributed to decrease of Se uptake and its assimilation to selenoproteins as well as enhancement of uptake of essential macroelements (K, P, S, Mg, N) resulting in an ameliorate the photosynthetic pigments and photosynthetic machinery, and hence improve the growth of rocket plants. In addition, Pearson correlation coefficient test was showed a negatively significant correlation between total Se content and each of biomass, leaf area, total pigments content and S content. In contrast a significant positive correlation between S and each of biomass, leaf area and total pigments content was detected.

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