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Original Research

Selenium Alleviates the Toxic Effects of Cadmium in Lupine (*Lupinus albus* L.)

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Abstract

This study aimed at investigating the effects of selenium (Se) against cadmium (Cd) toxicity on the plant growth, antioxidative enzyme activity, and Cd and Se content in lupine (*Lupinus albus* L.) plant and soil. The experiment was carried out under controlled conditions according to the factorial experiment design. Two doses of Cd (Cd_1:25 mg $kg^{-1}$and Cd_2:50 mg kg^{-1}) were applied in the soil. Three doses of Se (Se_1:2,5 mg kg^{-1} Se_2: 5 mg kg^{-1}, and Se_3:10 mg kg^{-1}) were applied to the Cd-treated pots. Two-way analysis of variance was conducted to explore the interactions between Cd and Se doses. The results showed that Cd_1 and Cd_2 applications alone caused a decrease in plant growth as well as increased oxidative stress due to shoot and root Cd content in plants and soil. Se_1 and Se_2 applications to Cd-treated pots caused an increase in shoot and root length, fresh and dry shoot weights, fresh and dry root weights. However, Se_3(10 mg kg^{-1}) application decreased shoot and root length, fresh and dry shoot weights, fresh and dry root weights. More importantly, application of Se (2,5 mg Se kg^{-1} and 5 mg Se kg^{-1}) is effective in improving the toxicity of cadmium increasing plant growth and diminishing shoot and root Cd, MDA content and DTPA-Cd content in lupine plant. We concluded that low and medium levels of Se alleviated the toxic effects of Cd in lupine. But high level of Se exerted negative influence on plant growth and oxidative stress under Cd toxicity. Moreover the effectiveness of selenium in reducing cadmium toxicity depends on the dose as well as the form of application.

Keywords: antioxidative enzymes, cadmium, selenium; plant growth, lupine (*Lupinus albus* L.)

Introduction

Cadmium (Cd) causes deterioration in photosynthesis, chlorophyll biosynthesis, and metabolic activities such as changes in carbohydrates metabolisms as well as significant changes in the uptake and transportation of nutrients. Cd toxicity causes a significant decrease in growth and development in the above-ground and root parts of plants [1, 2]. Oxidative stress, another phytotoxic effect of Cd, is the formation of reactive oxygen species (ROS). Plant cells obstruct the harmful effects of ROS with their antioxidant protection mechanism [3]. The implementation of mineral elements (e.g., iron, zinc, selenium, and silicon) to diminish Cd

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uptake and accumulation is also suggested as safe and effective method among agricultural practices that increases mineral uptake [4]. Similarly, Symanowicz et. al. [5] have reported that nitrogen fertilizer application did not exceed the limit values allowed in feedstuffs by Polish and European standards for the content of Cu, Zn, Ni, and Cr (except for Pb and Cd) in a legume plant (*Galega orientalis Lam.*). Selenium (Se) is not an essential element for plants indeed. However, Se application significantly decreases Cd uptake and accumulation in plant tissues [6, 7]. Foliar spraying with Silicon (Si) and Se contributed to Cd accumulation in rice cultivars [8]. The potential mechanism of Se that diminishes Cd accumulation in plant which is associated with deceleration of superoxide dismutase (SOD) and peroxidase (POD) activities is that Se reduces metal bioavailability in soil [6, 9]. Se application in Cd contaminated soils enhances cell wall binding capacity and the formation of Cd in less mobile forms [4]. The healing effect of Se is associated with the formation of Se-metal-complex which is non-toxic in Cd toxicity conditions. However, Se increases the production of α-tocopherol and SOD, both of which cause the removal of singlet oxygen in chloroplasts of plants exposed to Cd stress [10, 11]. Se has a significant effect on antioxidative enzymes [(SOD, ascorbate peroksidaz (APX), glutatyon reductase (GR) and guaiacol peroxidase (GPX)] which is one of the defense mechanisms of the plant under Cd stress conditions [12]. Cartes et al. [13] reported that low concentration Se decreases thiobarbituric acid reactive substances (TBARS) concentration and significantly changes antioxidative enzymes such as SOD and APX. Thus, Se attenuated oxidative stress caused by Aluminum (Al) in ryegrass. Se is a nonmetallic micronutrient that is beneficial to plants at very low concentrations. However, the optimum concentration varies with the plant species [14]. The effect low dosess of Se in plant growth such as antioxidants, stress relievers and inhibitors of the uptake of metals including Cd [15], Cr [16], Pb [17], As [18], Hg [19] etc. have been reported. SeNPs is known as an environmentally friendly and ecologically biocompatible approach to enhance crop production by alleviating biotic and abiotic stresses Selenium nanoparticles (SeNPs) have excellent potential application in the alleviation of heavy metal stress [20]. The protein content of the lupine plant was higher than that of other legumes. Lupine generally contains 5-20% fat, 30-40% fiber, and mainly antioxidants [21]. In studies on legumes, it has been found that plants in this group contain more Se than other plants [22]. In the current study, it was aimed at investigating the interection between Cd and Se applied to the lupine plant. To this end the plant growth, antioxidant enzyme activity, lipid peroxidation, plant root, and shoot and soil Cd and Se concentrations were assessed.

Material and Methods

Experiment Soil Characteristics

The soil material was taken from the study areas of the Faculty of Agriculture of Van Yüzüncü Yıl University. This soil is characterized by medium calcareous, alkaline pH, low organic matter, and low nitrogen (N). The zinc, iron and manganese content were low. The copper level was found to be sufficient. As can be seen in Table 1, exchangeable cations K, Ca, Na and Mg levels were found to be highly consistent with previous studies [23, 24].

Treatments and Experimental Design

The study was laid out in a completely randomized design (CRD) with factorial arrangements with three repetitions. The experiment was conducted with a total of 72 pots, 2.5 kg of soil was filled in each pot. Eight seeds were sown in each pot, and it was diluted to five plants after germination. Chemical fertilizers of 80 mg kg^{-1} phosphorus (P) as triple superphosphate, 200 mg kg^{-1} nitrogen (N) as ammonium nitrate and 50 mg kg^{-1} potassium (K) as potassium sulphate were added. Phosphorus and potassium fertilizers and half of the nitrogen fertilizer were applied to the soil

Table 1. Experiment soil characteristics.

	Unit	Experiment Soil
Texture		Sandy-Loam
pH (1/2.5)		8,75
Salt	dS m-1	0,27
Lime	%	10,47
Organic Material	%	0,80
Total N	%	0,04
P	mg kg^{-1}	1,70
Extractable with DTPA		
	mg kg^{-1}	
Fe		1,62
Zn		0,38
Cu		1,03
Mn		7,71
Exchangeable cations		
	me/100 g	
K		0,83
Ca		18,8
Mg		6,7
Na		3,7

before sowing. The other half of the nitrogen fertilizer was applied to the soil after seed germination**.** Cd of 25 mg kg^{-1} and 50 mg k^{g-1} doses as cadmium nitrate ($Cd(NO_3)_2.4H_2O$), Se of 2,5 mg kg^{-1}, 5 mg $kg^{-1}$and 10 mg kg^{-1} as sodium selenate (Na_2SeO_4) were applied in soil. Cadmium was applied at the seedling stage. Selenium was applied simultaneously with cadmium. Our experiment was carried out in a 2-month period with germination. Plant samples for enzymes were taken before harvesting. The harvest was made in the 8th week. Soil samples were taken in the period following the collection of plant samples. Cd, Se and combinations of these applications consisted of 12 applications. The applications are as follows: **1**-Cd_0 Se_0:Control, **2**-Cd_0 Se_1:2,5 mg kg^{-1}Se, **3**-Cd_0 Se_2:5 mg kg^{-1} Se, **4**-Cd_0Se_3:10 mg kg^{-1} Se, **5**-Cd_1Se_0:25mg kg^{-1}Cd, **6**-Cd_1Se_1:25 mg/kg Cd + 2,5 mg/kgSe,**7**-Cd_1Se_2:25 mg/kg Cd + 5 mg/kgSe, **8**-Cd_1Se_3:25 mg/kg Cd + 10 mg/kgSe, **9**-Cd_2+Se_0:50 mg/kg Cd, **10**-Cd_2Se_1:50 mg/kg Cd + 2,5 mg/kgSe, **11**-Cd_2Se_2:50 mg/kg Cd + 5 mg/kgSe, and **12**-Cd_2 Se_3:50 mg/kg Cd + 10 mg/kgSe.

Measurements of Antioxidative Enzymes and MDA Content in Plant

Superoxidedismutase (SOD) enzyme activity: The activity of SOD (EC1.15.1.1) was assayed by measuring it sability to inhibit the photochemical reduction of nitrobluetetra-zolium (NBT) following the method of Giannopolitis and Ries [25]. There action mixture (1 mL) included 50 mM phosphate buffer (pH 7.4), 13 mM methionine, 75 µM NBT, 0.1 mM EDTA, 2 µM riboflavin and 100 µL enzyme extract. There action was allowed to proceed for 15 minitues illuminated with fluorescent tubes. Absorbance of there action mixture was read at 560 nm. One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of photochemical reduction of NBT. SOD activity was expressed as U mg^{-1} protein.

Catalase (CAT) enzyme activity: CAT (EC1.11.1.6) activity was assayed by the decomposition of hydrogen peroxide (H_2O_2) according to [26]. A mixture of 0.05 M phosphate buffer (KH_2PO_4) and 100 mM H_2O_2 (pH:7) was prepared as there action solution. Absorbance change of the spectrophotometer was measured in a mixture of 2.5 ml reaction solution and 0.2 ml supernatantat 240 nm.

Ascorbate peroxidase (APX) enzyme activity: APX (EC1.11.1.1) activity was determined by the method of Nakano and Asada [27]. There action mixture (2 mL) contained 50 mM phosphate buffer (pH7.8), 0.1 mM EDTA, 0.3 mM ascorbate (AsA), 0.1 mM H_2O_2 and 100 µL enzyme extract. There action was initiated by addition of H_2O_2 and the oxidation rate of ascorbic acid was estimated by following the decrease in absorbance at 290 nm.

Lipid Peroxidation: The level of lipidperoxidation in plant leaves was determined by estimation of the thiobarbituric acid (TBA) reactive substances which was expressed as the malondialdehyde (MDA) concentration based on the method of Hodges et al. [28]. Briefly, a fresh leaf sample (0.2 g) was ground in 0.1% (w/v) TCA and the homogenate was centrifuged at 10.000 × g for 5 min. to 1 mL enzyme extract, 4 mL(TBA) [5% TBA (w/v) in 20% TCA (w/v)] was added. The mixture was heated at 100°C for 30 min and then cooled in an ice bath. After centrifugation at 10.000 × g for 10 min, the absorbance of the enzyme extract was measured at 532 nm. The value was corrected forthenon-specific absorption at 600 nm. Lipid peroxidation level was expressed as nmol MDA for medusing an extinction coefficient of 155 $mM^{-1}cm^{-1}$.

Determination of shoot and root Cd and Se concentrations: Dried plant shoot and root samples were digested with a mixture of HNO_3-$HClO_4$ acids and analysed for the concentration of Pb, Cd, Cr, and Zn by using atomic absorption spectrophotometer [29].

Determination of soil analyses: Soil pH; was determined potentiometrically by a "glass electrode" pHmeter in a 1:2.5 soil-water suspension [30]. Lime content of the soils was determined volumetrically by Scheibler calcimeter [31]. The amount of organic matter in the soil was determined according to the "Modified Walkley Black" method [32]. Exchangeable cations were extracted with 1N ammonium acetate [33]. The available micro elements were extracted with 0.05 M DTPA with a pH of 7.3 [34].

Statistical Analyses

The current research was a 4 x 3 randomized factorial design experimental study with three replicates. Using factorial design analysis of variance (ANOVA), the interaction effects between four levels of Se and three levels of Cd were evaluated separately for yield criteria, antioxidative enzyme activity, and heavy metal content in the plant. The posthoc analyses were conducted using the Duncan multiple group comparison test. The significance alpha level was held at $p<0.05$. The data were analyzed with SPSS Version 23.0 [35].

Results

Plant Growth

In the generalized linear models, the plant growth measurements were treated as dependent variable in each analysis separately. The two-way ANOVA analyses showed that F values for interactions between four doses of Se and three doses of Cd for shoot length, number of leaves, shoot fresh weight, shoot dry weight, root length, root fresh weight, and root dry weight were all statistically significant ($p<0.01$) (Table 2). The posthoc comparisons across twelve groups of interaction revealed consistent patterns of relationships

Table 2. Two-way ANOVAs for interaction effects of Se x Cd applications on plant growth parameters in lupine.

	Cadmium (Cd)			
	Selenium (Se)	Cd_0	Cd_1	Cd_2
	Se_0	12.08±0.29a	10.00±0.66c	9.58±0.63c
Shoot length	Se_1	11.75±0.25a	11.58± 0.14a	12.08±0.38a
(cm)	Se_2	11.08 ±0.95ab	11.92±0.63a	12.67±0.63a
	Se_3	9.72±0.68c	10.10±0.33c	10.28±0.26bc
Significant effects	*Selenium*	*Cadmium*	*Selenium x Cadmium*	
	F(3, 24) = 26.446;	*F(2, 24) = 0.908;*	*F(6, 24) = 8.578;*	
	$p<0.001$, $\eta2 = 0.768$	$p= 0.471$, $\eta2 = 0.070$	$p<0.001$, $\eta2 = 0.682$	
	Se_0	6.00±0.00a	5.48±0.26ab	4.90±0.66c
Number of leaves	Se_1	5.60±0.10ab	5.77±0.32a	5.83±0.22a
(per plant^{-1})	Se_2	5.03±0.35bc	5.72±0.19a	6.15±0.71a
	Se_3	4.97±0.29c	5.13±0.58bc	5.88±0.16a
Significant effects:	Selenium	Cadmium	Selenium x Cadmium	
	F(3, 24) = 1.972;	F(2, 24) = 1.730;	F(6, 24) = 5.364;	
	$p = 0.145$, $\eta2 = 0.198$	$p = 0.199$, $\eta2 = 0.126$	$p = 0.001$, $\eta2 = 0.573$	
	Se_0	3.00±0.055b	2.28±0.74d	2.04±0.08d
Shoot fresh weight	Se_1	3.11±0.18b	2.72±0.03c	2.90±0.13bc
(g plant^{-1})	Se_2	3.03±0.08b	2.93±0.095bc	3.49±0.25a
	Se_3	2.28±0.09d	2.10±0.28d	2.18±0.08d
Significant effects:	*Selenium*	*Cadmium*	*Selenium x Cadmium*	
	F(3, 24) = 90.377;	*F(2, 24) = 19.204;*	*F(6, 24) = 13.931;*	
	$p<0.001$, $\eta2 = 0.919$	$p< 0.001$, $\eta2 = 0.615$	$p < 0.001$, $\eta2 = 0.777$	
	Se_0	0.36±0.01a	0.23±0.006b	0.25±0.02b
Shoot dry weight	Se_1	0.34±0.006a	0.34±0.01a	0.32±0.02a
(g plant^{-1})	Se_2	0.34±0.01a	0.34±0.03a	0.36±0.02a
	Se_3	0.24±0.03b	0.22±0.03b	0.23±0.01b
Significiant effects:	Selenium	Cadmium	Selenium x Cadmium	
	F(3, 24) =81.787;	F(2, 24) =15.184;	F(6, 24) = 12.927;	
	$p<0.001$, $\eta2 = 0.911$	$p< 0.001$, $\eta2 = 0.559$	$p< 0.001$, $\eta2 = 0.764$	
	Se_0	17.04±0.05a	12.84±1.62c	12.94±1.79c
Root length	Se_1	17.17±2.52a	17.27±0.49 a	16.67±0.58ab
(cm)	Se_2	16.83±1.04a	17.60±0.96a	18.67±0.58a
	Se_3	14.63±0.55bc	13.69±1.01c	15.11±0.46ab
Significant effects:	*Selenium*	*Cadmium*	*Selenium x Cadmium*	
	F(3, 24) = 19.909;	*F(2, 24) = 2.483;*	*F(6, 24) = 4.395;*	
	$p<0.001$, $\eta2 = 0.713$	$p = 0.105$, $\eta2 = 0.171$	$p = 0.004$, $\eta2 = 0.524$	
	Se_0	0.87±0.006a	0.54±0.11b	0.52±0.02b
Root fresh weight	Se_1	0.83±0.02a	0.80±0.09a	0.92±0.13a
(g plant^{-1})	Se_2	0.82±0.05a	0.85±0.04a	0.78±0.14a
	Se_3	0.50±0.06b	0.59±0.02b	0.63±0.06b

Table 2. Continued.

Significant effects:	*Selenium*	*Cadmium*	*Selenium x Cadmium*	
	F(3, 24) = 27.446;	*F(2, 24) = 2.143;*	*F(6, 24) = 7.967;*	
	p<0.001, η2 = 0.774	*p< 0.139, η2 = 0.152*	*p< 0.001, η2 = 0.666*	
	Se_0	0.07±0.002a	0.04±0.006b	0.04±0.009b
Root dry weight	Se_1	0.07± 0.006a	0.06±0.004ab	0.07±0.006a
(g plant^{-1})	Se_2	0.07±0.008a	0.07±0.005a	0.07±0.007a
	Se_3	0.05±0.007b	0.05±0.003b	0.06±0.009b
Significant effects:	*Selenium*	*Cadmium*	*Selenium x Cadmium*	
	F(3, 24) =20.106 ;	*F(2, 24) = 8.314;*	*F(6, 24) = 4.587;*	
	p<0.001, η2 = 0.715	*p = 0.002, η2 = 0.409*	*p= 0.003, η2 = 0.534*	

Note. a, b, c, d:significant post-hoc difference between applications indicated with different lowercase letters ($p< 0.05$).

between different doses of Se and Cd in terms of plant growth in lupine. Either Cd_1 alone or Cd_2 alone caused a decrease in shoot length, number of leaves, shoot fresh weight, shoot dry weight, root length, root fresh weight, and root dry weight compared to control (Se_0Cd_0). However, either Se_1 alone or Se_2 alone did not result in significant differences in plant growth measurements from the Se_0Cd_0. However, Se_3 alone significantly decreased in plant development**.** More importantly, under Cd toxicity conditions, both Se_1 and Se_2 caused an increase in shoot lenght, shoot fresh and dry weight, root lenght, root fresh and dry weight of lupine. The findings are presented in Table 2.

Antioxidative Activity in Lupine (*Lupinus albus* L.)

To investigate the Se x Cd interaction effects on antioxidative enzyme activity in the lupine plant, 4 x 3 factorial design two-way ANOVAs were performed. In theANOVAs, antioxidative enzymes of SOD, CAT, APX and MDA were treated as dependent variables. The two-way ANOVA analyses showed that the F values computed for Se and Cd interactions for all antioxidative enzymes in the lupine plant were statistically significant (p<0.01). The posth occomparisons were performed using the duncan multiple comparison test (Table 3). The posthoc comparisons across twelve groups of interaction revealed significant and consistent patterns of relationships between different doses of Se and Cd in terms of antioxidative activity in response to oxidative stress in the lupine. Similarly, Cd_1 and Cd_2 alone applications resulted in a significant increase in SOD, CAT, APX and MDA as well. Se_1 alone application caused increased SOD enzyme activity compared to Cd_1 alone application. Incontrast, either Se_1 or Se_2 applications under various levels of Cd toxicity significantly decreased SOD, APX, CAT enzyme activities and MDA content in lupine. In sharp contrast, Se_3 alone application caused a significantly increase in MDA activities compared to Se_0Cd_0 in thelupine. Findings are presented in Table 3.

Cd and Se Content in the Shoot and Root of Lupine (*Lupinus albus* L.), and Soil

To investigate the interaction effects of various levels Se and Cd on heavy metal content in plant and soil, 4 x 3 factorial design two-way ANOVAs were performed. In the ANOVA analyses, measurements of Se and Cd content in the root and shoot of the plant as well as in soils were treated as dependent variables. The two-way ANOVA analyses showed that F values of interactions between Se and Cd contents in shoot and root of lupine as well as in soil were statistically significant (p<0.01). The posthoc comparisons across twelve groups of interaction revealed significant relationships between different doses of Se and Cd in terms of Cd and Se content in the shoot and root of the plant as well as in the soil. As expected, Cd_1 and Cd_2 alone applications resulted in a significant increase in Cd content in shoots and roots of plant and soil. Either Se_1 or Se_2 applications in both levels of Cd applications significantly decreased Cd content in the shoot as well as in the soil. Se_2 application, in both levels of Cd, resulted in decreased Cd content in the root of plant. However, Se_3 application did not cause a substantial decrease in Cd content in the shoot and root of lupine as well as in the soil. Increasing doses of Se resulted in an increase in Se content in the shoot and root of plant and soil in Cd-free conditions. Findings are presented in Table 4.

Discussion

Plant Growth

Heavy metal stress may cause changes in plant morphology, physiology, growth, and yield. Cd is

Table 3. Two-way ANOVAs for interaction effects of Se x Cd applications on antioxidative enzymes activity and MDA contentin lupine.

	Cadmium (Cd)			
	Selenium (Se)	Cd_0	Cd_1	Cd_2
	Se_0	52.68±7.45f	81.83±8.16e	160.33±2.52b
SOD	Se_1	79.58±4.02e	122.83±8.96c	57.37±1.95f
Unit g^{-1}F.W.	Se_2	83.92±6.45e	63.80±10.30f	66.43±5.57ef
	Se_3	450.00±0.00a	100.71±20.75d	66.08±9.28ef
Significiant effects:	*Selenium*	*Cadmium*	*Selenium x Cadmium*	
	F(3, 24) = 436.138;	*F(2, 24) =305.855;*	*F(6, 24) = 539.932;*	
	p <0.001, η2 = 0.982	*p<0.001, η2 =0.962*	*p< 0.001, η2 = 0.993*	
	Se_0	0.01±0.00c	0.18±0.04b	0.40±0.06a
CAT	Se_1	0.02±0.006c	0.02±0.00c	0.06±0.02c
mmol g^{-1}F.W.Min^{-1}	Se_2	0.03±0.00c	0.03±0.00c	0.03±0.02c
	Se_3	0.14±0.006b	0.05±0.02c	0.15±0.01b
Significiant effects:	*Selenium*	*Cadmium*	*Selenium x Cadmium*	
	F(3, 24) = 107.168;	*F(2, 24) =75.931;*	*F(6, 24) = 53.881;*	
	p <0.001, η2 = 0.931	*p<0.001, η2 = 0.864*	*p< 0.001, η2 = 0.931*	
	Se_0	0.27±0.025d	2.68± 0.43b	6.05±0.50a
APX	Se_1	1.44±0.19c	3.62±0.45b	3.04±0.07b
mmol g^{-1}F.W.Min^{-1}	Se_2	0.67±0.13d	0.36±0.10d	2.24±0.10b
	Se_3	1.24±0.12c	2.96±1.16b	2.43±0.09b
Significiant effects:	*Selenium*	*Cadmium*	*Selenium x Cadmium*	
	F(3, 24) = 36.440;	*F(2, 24) = 111.878;*	*F(6, 24) = 28.706;*	
	p<0.001, η2 = 0.820	*p<0.001, η2 = 0.903*	*p< 0.001, η2 = 0.878*	
	Se_0	4.84±0.070d	7.89±0.77b	8.09±0.08b
MDA	Se_1	5.48±0.426d	7.02±0.81c	6.17±0.33c
nmol g^{-1}F.W.	Se_2	6.69±0.260c	6.57±0.44 c	6.68±0.48c
	Se_3	10.78±0.359a	7.63±0.25b	7.46±0.24b
Significiant effects:	*Selenium*	*Cadmium*	*Selenium x Cadmium*	
	F(3, 24) = 52.601;	*F(2, 24) =1.756 ;*	*F(6, 24) = 38.539;*	
	p <0.001, η2 = 0.868	*p<0.194, η2 = 0.128*	*p< 0.001, η2 = 0.906*	

Note. a, b, c, d, e, f:significant post-hoc difference between applications indicated with different lowercase letters ($p< 0.05$)

easily taken up by the plant due to its high mobility. It adversely influences the development of the plant by reducing growth. Specifically, Cd toxicity seriously affects plant growth parameters due to its effects on nitrogen and carbohydrate metabolisms in the plant [36, 18]. It was indicated that Cd damages photosynthetic activy, seedling germination, plant water relations, plant biomass mechanisms, crop yield and inhibits the uptake of essential nutrients from the soil [37-39]. Cd not only disrupts the plant's metabolism but also interferes with its normal development by disrupting nutrient uptake [40]. It was determined that the N, Ca, Mg, and P contents of the parts of the alfalfa plant decreased under cadmium toxicity conditions [41]. In this study, it was found that 25 and 50 mg Cd kg^{-1} treatments caused a decrease in shoot length, number of leaves, shoot fresh and dry weight, root length, root fresh and dry weight of the lupine plant (Table 2). These findings were consistent with the previous research. In a study carried out with lupine plant, 45 µM Cd applied in hydroponic culture caused a significant decrease in both root and shoot dry weight, nodulation and total N content [42]. Cd toxicity caused reduced root length, and dry mass and increased root diameter in tomatoes as well [43]. It has been

Table 4.Two-way ANOVAs for interaction effects of Se x Cd treatments on Cd and Se content in plant and soil (mg kg^{-1}).

Cadmium (Cd)				
	Selenium (Se)	Cd_0	Cd_1	Cd_2
	Se_0	0.00±0.00 h	141.18±7.49c	194.20±3.26a
Cd (shoot)	Se_1	0.00±0.00 h	98.24±2.68e	178.03±3.72b
	Se_2	0.00±0.00 h	65.36±3.29f	46.56±3.83g
	Se_3	0.00±0.00 h	113.67±4.91d	185.00±15.59ab
Significiant effects:	*Selenium*	*Cadmium*	*Selenium x Cadmium*	
	F(3, 24) =307.984;	*F(2, 24) = 2263.424;*	*F(6, 24) = 124.567;*	
	p <0.001, η2 = 0.975	*p < 0.001, η2 = 0.995*	*p< 0.001, η2 = 0.969*	
	Se_0	0.00±0.00h	1.76±0.13h	1.64±0.16h
Se (shoot)	Se_1	43.79±0.88g	61.15±2.37f	58.53±3.57f
	Se_2	63.18±2.59f	121.65±4.42d	109.92±4.92e
	Se_3	173.16±8.12c	255.93±10.78a	182.81±9.07b
Significiant effects:	*Selenium*	*Cadmium*	Selenium x Cadmium	
	F(3, 24) = 2394	F(2, 24) = 173.7;	F(6, 24) =53.23;	
	p <0.001, η2 = 0.997	p < 0.001, η2 = 0.935	p< 0.001, η2 = 0.930	
	Se_0	0.00±0.00h	15.41±0.34d	24.21±0.70ab
Cd (root)	Se_1	0.00±0.00h	15.58±1.14d	22.69±2.06b
	Se_2	0.00±0.00h	13.03±1.29e	17.88±0.98c
	Se_3	0.00±0.00h	15.95±1.36d	25.41±0.72a
Significiant effects:	*Selenium*	*Cadmium*	*Selenium x Cadmium*	
	F(3, 24) =22.78;	*F(2, 24) = 1702.5;*	*F(6, 24) = 9.124;*	
	p <0.001, η2 = 0.740	*p < 0.001, η2 = 0.993*	p< 0.001, η2 = 0.695	
	Se_0	0.02±0.00h	0.00±0.00h	0.00±0.00h
Se (root)	Se_1	66.39±1.62f	9.39±2.90g	77.69±5.82e
	Se_2	132.73±6.93d	3.63±0.27h	128.64±6.22d
	Se_3	220.79±0.17 a	199.96±12.06b	174.31±2.70c
Significiant effects:	*Selenium*	*Cadmium*	*Selenium x Cadmium*	
	F(3, 24) = 2686.452;	*F(2, 24) = 382.174;*	*F(6, 24) = 179.167;*	
	p<0.001, η2 = 0.997	*p<0.001, η2 = 0.970*	*p<0.001, η2 = 0.978*	
	Se_0	0.01±0.003h	24.20±1.33e	53.43±0.26b
Cd (soil)	Se_1	0.32±0.061h	21.18±0.80f	32.21±3.12d
	Se_2	0.15±0.006h	13.06±0.17g	41.95±3.08c
	Se_3	0.17±0.006h	25.54±0.52e	56.66±3.69a
Significiant effects:	*Selenium*	*Cadmium*	*Selenium x Cadmium*	
	F(3, 24) = 74.880;	*F(2, 24) = 2138.182;*	*F(6, 24) = 41.750;*	
	p<0.001, η2 = 0.903	*p<0.001, η2 = 0.994*	*p<0.001, η2 = 0.913*	
	Se_0	0.02±0.00i	0.00±0.00i	0.00±0.00i
Se (soil)	Se_1	4.51±0.54h	6.48 ±0.43f	4.71±0.39h
	Se_2	19.82±0.21c	5.45 ±0.10g	8.56±0.58e
	Se_3	21.41±0.25b	29.64 ±0.30a	17.72±0.86d

Table 4.Continued.

	Selenium	*Cadmium*	*Selenium x Cadmium*
Significiant effects:	*F(3, 24) = 5452.494;*	*F(2, 24) = 271.428 ;*	*F(6, 24) = 506.309;*
	p<0.001, η2 = 0.999	*p<0.001, η2 = 0.958*	*p<0.001, η2 = 0.992*

Note: a, b, c, d, e, f, g, h, i:significant post-hoc difference between applications indicated with different lowercase letters ($p< 0.05$).

suggested that Se treatment in Cd contaminated soils may be effective. In this study, plant height, plant fresh weight, plant dry weight, root length, root fresh weight, and root dry weight increased significantly with 2.5 and 5 mg kg^{-1} Se (the form of Selenate) applications under 25 and 50 mg Cd kg^{-1} applied conditions. However, the application of 10 mg Se kg^{-1} didn't cause improvement in plant growth in Cd toxicity conditions and exerted an impairment effect on plant development even in Cd-free conditions. More specifically, Selenate has a crucial role in curing heavy metal toxicity when administered at low doses [44, 45]. Mozafariyan et al. [46] reported that low doses Se application increased the fruit number and total yield of pepper under Cd stress. The number of fruits, fruit length and diameter, single fruit weight, and total yield of cucumber increased in mid-level applied selenium under cadmium and lead stress [47]. Qi et al. [48] reported that the toxic heavy metal effect on plant growth of radish in Cd-contaminated soil was alleviated with low dose Se treatment. However, high doses of Se inhibited growth of radish. Spraying Se could also recover tomato growth under Cd stress and increase the dry weights of roots, fruits, and leaves [49]. Sepehri et. al. [50] determined that Selenate application to garlic plant caused an increase of root and shoot fresh weight by about 6.8% and 14.59% under cadmium applied conditions selenate appliaction improved the net photosynthesis, stomatal conductance, intercellular carbon dioxide (CO_2) concentration, and chlorophyll content in wheat plants under Cd stress [51]. Plants uptake selenate by the sulfate transport system in the plasma membrane of the roots [52]. Qi et.al. [53] reported that both selenium nanoparticles (SeNP's) and sodium selenite (SeO_3^{2-}) have positive effect on the growth of *Brassica napus* L. Under Cr stress, the exogenously applied Se significantly recovered the impairment in photosynthesis-related parameters; chlorophyll a, chlorophyll b, photosynthetic rate, plant growth, in nutrient uptake and improved the essential amino acids (EAAs) levels [16].

Antioxidative Enzymes in Lupine (*Lupinus albus* L.)

Under Cd toxicity conditions, reactive oxygen radicals are produced as a result of oxidative stress. The plant's antioxidant enzymes such as SOD, GR, CAT, and APX and non-enzymatic mechanism reduce or oxidize free radicals, thereby ending the destructive effect of radicals [54, 55]. Cd application in soybean plants increased lipid peroxidation, hydrogen peroxide content. In SOD, POD and CAT enzyme activities caused an increase in Cd treatment [56]. Similarly, in the study carried out with the lupine plant, Cd and Pb caused an increase in antioxidative enzyme activity [57]. In keeping with the previous findings, the present study showed that 25 mg Cd kg^{-1} and 50 mg Cd kg^{-1} applications resulted in increased SOD, CAT and APX enzyme activities, and MDA content in lupine plant (Table 3). Cd binds irreversibly to SH groups in enzyme and membrane proteins. With Se application, enzymes with metal-protein complexes in chloroplasts are positively influenced and the non-toxic Se-Metal complex that supports the activation of protein enzymes exerts a protective effect on the plant. Therefore, Se has a positive effect on enzymatic and non-enzymatic antioxidants [58, 59]. In this study 2.5 and 5 mg Se kg^{-1} applied caused a decrease in MDA activity under Cd toxicity conditions. However, 2.5 and 5 mg, Se kg^{-1} applications in two levels of Cd significantly decreased SOD, APX, CAT enzyme activities and MDA content in lupine (Table 3). Plants have different tolerance methods for detoxification effect on Cd toxicity. Saidi et al. [12] reported that exogenous Se caused an increase in CAT, APX, and glutathione reductase enzyme activities and a decrease in MDA content under Cd toxicity in the sunflower plant. It is well-established that Se protects the plant against oxidative stress caused by Cd toxicity by strengthening the antioxidative defense mechanisms of the plant. Supplementation of selenium to cadmium-treated plants (Cd + Se), to relieve Cd toxicity and repair damaged cells enhanced the activity of antioxidative in tomato [60]. It has been reported that the Se treatment in plants that exposed to Cd improves stress conditions and causes an increase in α-tocopherol that regulates ROS metabolism and decreases MDA and H_2O_2 levels [40, 55]. In the cucumber plant, Se caused an increase in the secretion of some specific proteins under Cd stress conditions [61]. SeNP are effective alternative for alleviating Cd stress. SeNP ameliorate Cd stress due to more potentially curtailing ROS production [53]. Se might be able to down regulate Cd-induced oxidative damages through the inhibition of ROS production and indirectly by regulation of antioxidative system including GSH biosynthesis. The regulation of ROS levels by Se may be a key mechanism for counteracting environmental stress in plants [62].

Content of Cd and Se in Lupine (*Lupinusalbus* L.)

In a heavy metal study carried out in white lupine, it was found that a significant part of the applied Cd retained in the roots of the plant [42]. In this study, Cd applications increased the content of Cd in root and shoot of lupine. However, the shoot Cd content was found to be higher than the root Cd content (Table 4). In Cd-free conditions, Se application as sodium selenate also caused an increase in root and shoot Se content (Table 4). Zornoza et al. [42] identified that Se applied at increasing levels increased Se content in both the leaf and root parts of the lupine plant. Similarly, increasing levels of Se application as sodium selenate in the bean plant caused an increase in Se content in the root, stem, and leaf parts [63]. In the current study, 2.5 mg Se kg^{-1} and 5 mg Se kg^{-1} levels caused a decrease in Cd content in both shoot and root parts of the lupine plant. Given the two doses of Cd treatments, with the application of 2,5 and 5 mg·Se kg^{-1} in the form of selenate, the Cd content in shoot of lupine decreased 30,82%, 8,25%, 53,98% and 75,82% respectively (Table 4). The concentration of Cd in the shoots of radish decreased by 20.39% and 5.34% with the application of 1 and 2.5 mg·kg^{-1} selenate [48]. Lin et al. [9] reported that Se application decreased Cd content in rice. Se significantly reduced Cd uptake in roots and leaves. Se application reduced shoot Cd content in rice [64] and radish [65]. These findings can be best explained by the antagonistic interaction between Cd and Se [66]. However, considering the higher doses of Se, direction of the interaction between Cd and Se becomes dose-dependent [67]. Foliar spraying with 5 μM Se diminished the Cd content in the shoots and roots of Chinese cabbage [68]. Se application decreased Cd content in wheat tissue and Cd uptake. Therefore, Cd concentration diluted in tissue due to increased plant biomass [69, 70]. When Se is applied at an appropriate dose, it increases the durability of the cell wall because it increases the formation of lignin in the plant. That causes decreased Cd diffusion in the roots as well as Cd accumulation [71]. Therefore reducing the metal content of Se causes an increase in growth in the plants.

Content of Cd and Se in Soil

Cd is a heavy metal that can cause serious health problems for human beings and other living organisms due to its high mobility in the soil, root, and seed systems. Given that Cd is widely deposited in the edible parts of plants, growing plants in polluted soils can cause this heavy metal join to the food chain [72]. In this study, 25 mg Cd kg^{-1} and 50 mg Cd kg^{-1} levels of Cd treatments to the soil resulted in an increase in the content of DTPA-Cd (Table 4). In Cd-free conditions, Se applications in the form of Na_2SeO_4 increased DTPA-Se content directly proportionate to increased levels of Se. However, 2.5 mg Se kg^{-1} and 5 mg Se kg^{-1} in Cd-contaminated soils in the form of Na_2SeO_4 caused a decrease in DTPA-Cd content (Table 4). Huang et al. [73] asserted that Na_2SeO_4 application increases soil pH that, in turn, decreases soil Cd bioavailability and mobility. This process results in low retainment of Cd in plants. It was reported that Na_2SeO_4 and selenite (Na_2SeO_3) are reduced to Se^{-1} when the Se: Cd ratio is greater than the threshold value. Thus, the bioavailability of Cd in the soil and accumulation in the plant will decrease [74]. Liu et al. [75] find that applications including a combination of Se with Cd could reduce the amount of Cd in soil solution. Therefore, Cd bioavailability and Cd uptake by plants might be decreased by Se treatments. External application of Se can cause the reduction of Se by soil microorganisms. That is, SeO_4^{2-} or SeO_3^{2-} can be reduced to SeO or Se^{2-} This may help formation of the $CdSeO_3$ compound and reduce Cd toxicity in soil [73, 76].

Conclusions

The application of 2.5 and 5 mg kg^{-1} of Se is effective in improving the toxicity of cadmium increasing plant growth and diminishing shoot and root Cd, MDA content and DTPA-Cd content in lupine plant. Therefore, it was concluded that low and medium levels of Selenate can be promote to alleviate the toxic effects of Cd in lupine. Moreover the effectiveness of selenium in reducing cadmium toxicity depends on the dose as well as the form of application.

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Conflict of Interest

We declare that there is no conflict of interest in the planning, execution and writing of the article.

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