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Research article

ALLEVIATION THE INHIBITORY EFFECT OF MERCURY AND CADMIUM ON PRODUCTION OF *VICIA FABA* PLANT BY APPLICATION OF SELENIUM

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ABSTRACT: The present work aimed to investigate the effect of $HgCl_2$, $CdCl_2$ (50, 200 µg/ml) and SeO_2 (0.4, 2.6 µg/ml) singly or in mixtures on yield parameters, biochemical analysis of seeds and protein pattern of *Vicia faba* L. plants (cv Sakha 1).

Heavy metal salts caused a reduction in production and yield parameters as compared to control. Applying selenium dioxide was highly significant attenuated the sever drop of yield parameters. Applying low selenium dioxide to stressed plants increased both sucrose and starch contents to a great extent which more appeared at lower than higher heavy metal levels, but the adverse effect was observed with high selenium dioxide which more pronounced at higher than lower levels of heavy metal salts as compared to single heavy metal salts.

The results of protein pattern showed considerable effects of heavy metal stress on protein bands. Selenium dioxide induced the synthesis of new bands and increased the intensity of the original protein bands.

Key words: Selenium, Mercury, Cadmium, Soluble protein, Reducing sugar, Sucrose, Starch, Yield parameters, Protein pattern, *Vicia faba*

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INTRODUCTION

Heavy metals are naturally present in soils and also, large amounts of heavy metals have accumulated and continue to accumulate in the biosphere due to mining, manufacturing, agricultural and municipal waste disposal practices [1]. Taking into account their concoction and physical properties three distinctive atomic systems of heavy metal poisonous quality can be recognized: (a) creation of responsive oxygen species via autoxidation and Fenton response; this response is normal for move metals, for example, iron or copper, (b) hindering of vital useful gatherings in biomolecules, this response has for the most part been accounted for non-redox-receptive substantial metals, for example, cadmium and mercury, (c) removal of key metal particles from biomolecules; the last response happens with various types of heavy metals. Introduction of plants to non-redox receptive metals additionally brought about oxidative anxiety as demonstrated by lipid peroxidation, H_2O_2 gathering, and an oxidative burst. Cadmium and some different metals created a restraint of antioxidative chemicals, particularly of glutathione reductase [2].

Heavy metals make a significant contribution to natural contamination as a consequence of human exercises [3]. Raised centralizations of both key and trivial overwhelming metals in the dirt can prompt lethality manifestations and development restraint in many plants [4]. What's more, an abundance of overwhelming metal may fortify the arrangement of free radicals and responsive oxygen species, may be bringing about oxidative anxiety [5].

Selenium is an essential micronutrient and has important benefits for animal and human nutrition. At high dosage, however, it may be toxic to animal [6,7] and to humans [8]. The chemistry of selenium has been reviewed extensively by several authors [9,10]. It is metalloid with an atomic weight of 78.96. Selenium shares many similar chemical properties with sulfur (S) [9].

Selenium can exist in five valence states, elemental Se (0), selenide (2^-), thioselenate (2^+), selenite (4^+), and selenate (6^+) [9]. However selenate is accumulated in plant cells against its likely electrochemical potential gradient through a process of active transport [11]. Selenate readily competes with the uptake of sulfate and it has been proposed that both anions are taken up via sulfate transporter in the root plasma membrane [12].

The translocation of selenium from root to shoot is reliant on the type of selenium supplied. Selenate is transported more effortlessly than selenite, or natural Se, for example, SeMet. [13] demonstrated that the shoot Se/root Se proportion extended from 1.4 to 17.2 when selenate was supplied yet was just 0.6 to 1 for plants supplied with SeMet and under 0.5 for plants supplied with selenite. Ref. [12] exhibited that inside of 3 h, half of the selenate taken up by bean plant roots moved to shoots, though on account of selenite, the greater part of the Se stayed in the root and just a little division was found in the shoot.

The reason why selenite is poorly translocated to shoots may be because it is rapidly converted to organic forms of Se such as SeMet [13], which are retained in the roots.

The distribution of selenium in various parts of the plant differs according to species, its phase of development, and its physiological condition [14]. Distribution of selenium in plants also depends on the form and concentration of selenium supplied to the roots and on the nature and concentration of other substances, especially sulfate, accompanying the selenium [13].

In spite of the fact that selenium is a key follow supplement vital to people and most different creatures as a cell reinforcement, lethality happens at high fixations because of supplanting of sulfur with selenium in amino acids bringing about off base collapsing of the protein and thusly nonfunctional proteins and catalysts [15].

Beans (*Vicia faba*) are viewed as the first vegetable harvest in Egypt of the arable territory. Aggregate yield and utilization as green and dry seeds are devoured in human food in light of the fact that the plant has abnormal amounts of protein (18%), sugars (58%), vitamins and different minerals. Notwithstanding the change of soil composition and its fruitfulness, the plant seeds are considered as a profitable hotspot for vitality and proteins.

In this way, the principle goal of this study was to evaluate the role of selenium dioxide to reduce the pollution of mercury and cadmium on production and biochemical components of *Vicia faba* seeds.

MATERIALS AND METHODS

1. Plant material

Faba bean seeds (*Vicia faba* L. cv. Sakha 1) were obtained from Sakha Agricultural Research Station, Kafr-Elsheikh, Egypt. The healthy seeds were selected for apparent uniformity of size and shape, and surface sterilized (2.5% Clorox for 5 min.) and rinsed thoroughly in distilled water.

2. Pot experiment

Sand - clay soil (½ v/v) was used, the soil was mixed thoroughly to assure complete and uniform distribution (25 cm diameter, 35 cm depth, 6 Kg soil pot $^{-1}$).

Faba seeds were divided into four groups, the first one was untreated soil as a control, the second one was treated with the different concentrations of metals salt solution mentioned before (Hg Cl_2, Cd Cl_2 and Se O_2) separately, the third one was treated with 50 Hg Cl_2+0.4 Se O_2, 200 Hg Cl_2+0.4 Se O_2,50 Hg Cl_2+2.6 Se O_2 and 200 Hg Cl_2+2.6 Se O_2 µg ml^{-1} and the fourth one was treated with 50 Cd Cl_2+0.4 Se O_2, 200 Cd Cl_2+0.4 Se O_2, 50 Cd Cl_2+2.6 Se O_2 and 200 Cd Cl_2+2.6 Se O_2 µg ml^{-1}. Five seeds were sown per pot. Each treatment was carried out in four replicates. The sowing date was November 2009 and experiment was conducted for about four months. Pots were irrigated with the different concentrations of metals salt solution, to 80% of field capacity level, every two weeks.

Nitrogen-phosphorus fertilizers were applied at rates of one gram of urea/pot and 2.3 gram of super - phosphate/pot, respectively. Phosphorus was added during soil preparation (i.e., before sowing). Nitrogen was applied after 5 weeks of sowing.

Measurements

At 120- day old, plants were collected to determine yield parameters (number of pods/plant, fresh weight of pods/plant, number of seeds/pod, fresh weight of seeds/pod and fresh weight of 1000 seeds), Biochemical analysis of seeds were estimated as carbohydrates were estimated quantitatively using Ref. [16] with some modifications was done by Ref. [17] and starch can be measured quantitatively. Soluble proteins were assayed according to Ref. [18].

Detection of protein pattern in seeds by the method of Ref. [19] with slight modifications was adopted to use in the present study. Data obtained were analyzed statistically to determine the degree of significance between treatments. The method of two ways analysis of variance (ANOVA; factorial) was applied for all data. The F-value was determined between means [20].

RESULTS AND DISSCUSION

Figure 1 and Figure 2 show the yield parameters and biochemical analysis of *Vicia faba* at 120-day old it found that, heavy metal salts caused a reduction in all yield criteria compared to control, particularly at high heavy metal salts level. Concerning, the seed soluble protein, reducing sugar, sucrose and starch contents were decreased with heavy metal which more pronounced at higher than lower levels as compared to control. In this connection, Ref. [21] reported that soil contamination with heavy metals will lead to lose in agricultural yield and hazardous health effects as they enter into the food chain. Also, Ref. [22] showed that the visual non-specific symptoms of heavy metal toxicity on plant are inhibition of root growth and seed weight.

Accordingly, Ref. [23] showed that the application of Hg caused a significant lower *Oryza sativa* tiller formation from the control in clay loam and sandy clay loam soil started at 1.50 and 1.00 mg kg^{-1} soil treatment, respectively. Grain yield gradually decreased from control to the highest rate of Hg application indicating unfavorable effects of Hg on grain filling process.

The decrease in grain yield, straw of rice might be the results of physiological disorders by accumulated Hg in plants such as reduction of chlorophyll, decrease of photosynthesis and related enzyme activities and disturbances in mineral nutrition finally leading to lower growth and yield [24,25]. Ref. [24] reported that the interactions between Hg and other nutrients may lead to changes in the nutrient content and physiological disorders as well as reduction of growth and yield. Ref. [26] reported that the growth and development of oats grown on Hg contaminated soil was also strongly inhibited. However, Ref. [27] decided that decrease in the number of tomato flowers was seen in all Hg treated samples as increasing in their level of metal, when compared to control.

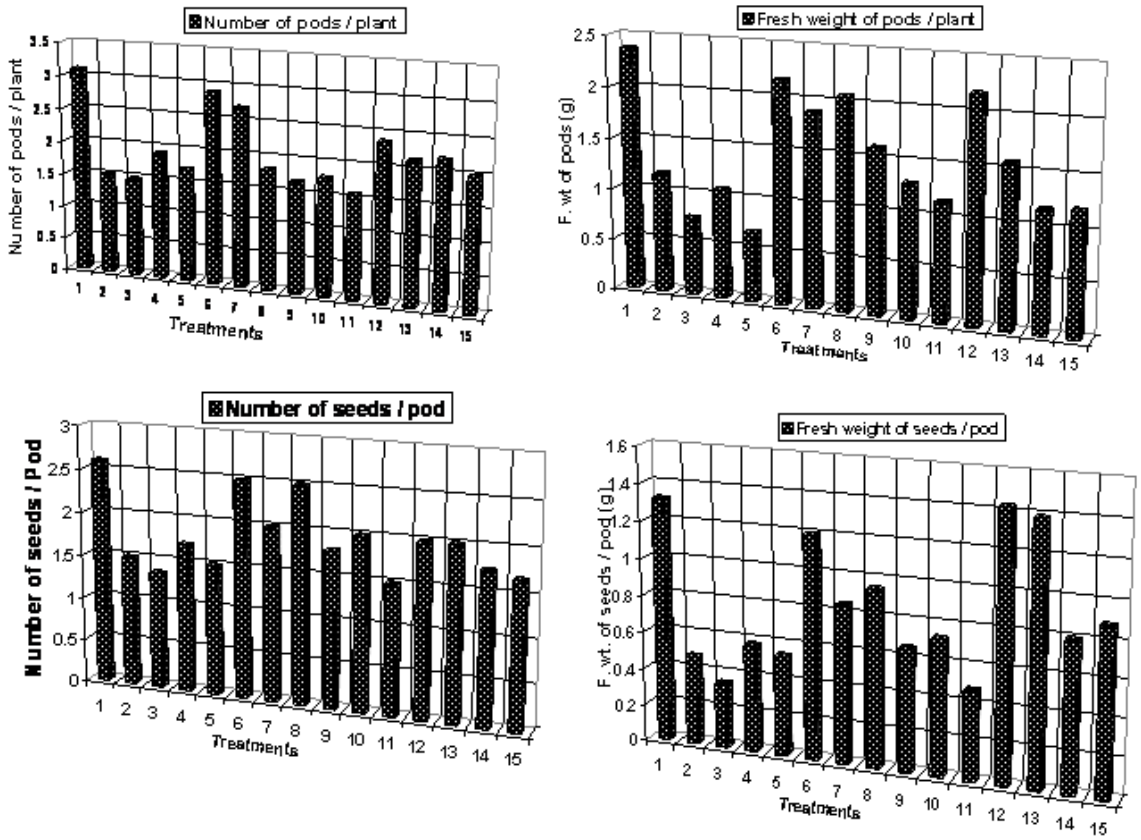
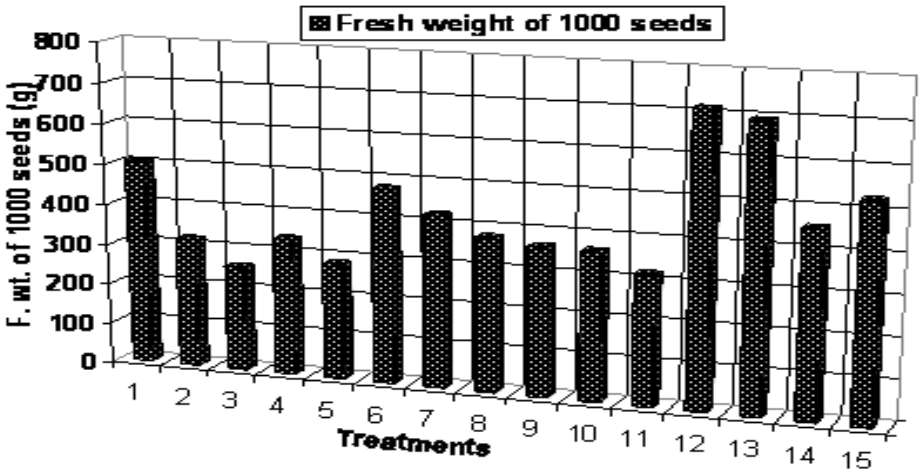
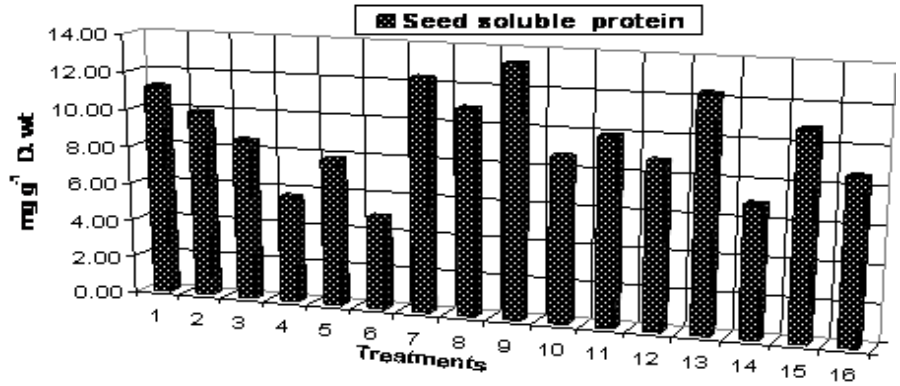
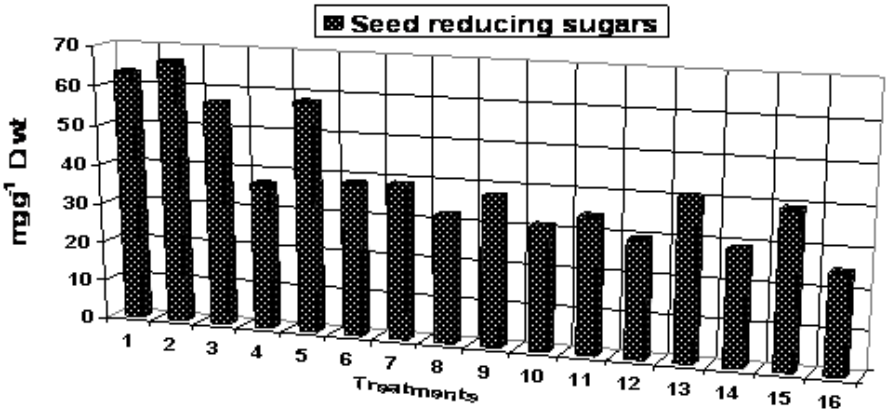
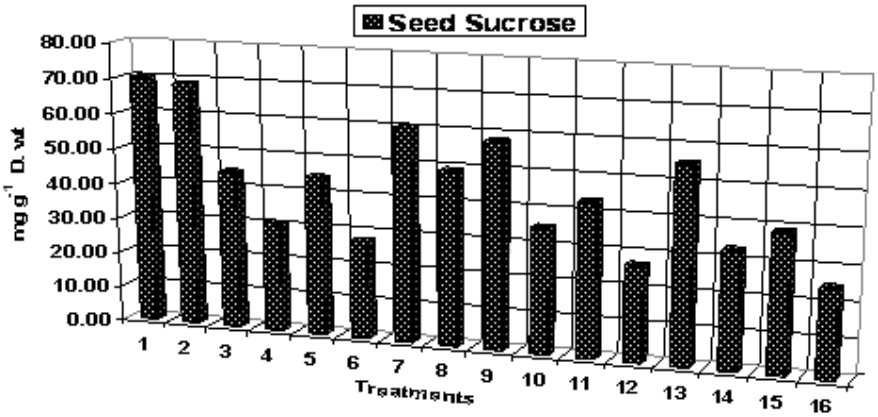


Figure 1: Yield parameters of *Vicia faba* at 120-day old as affected by SeO_2 (0.4, 2.6 µg/ml), $HgCl_2$ and $CdCl_2$ (50, 200 µg/ml), and their mixtures. 1: Control, 2: Hg 50 µg ml^{-1}., 3: Hg 200 µg ml^{-1}, 4: Cd 50 µg ml^{-1}, 5: Cd 200 µg ml^{-1}, 6: Se 0.4 µg ml^{-1}, 7: Se 2.6 µg ml^{-1}, 8: Hg 50 Se 0.4, 9: Hg 200 Se 0.4, 10: Hg 50 Se 2.6, 11: Hg 200 Se 2.6, 12: Cd 50 Se 0.4, 13: Cd 200 Se 0.4, 14: Cd 50 Se 2.6, 15: Cd 200 Se 2.6.


Fresh weight of 1000 seeds
F. wt. of 1000 seeds (g)
800
700
600
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Treatments


Seed soluble protein
mg g-1 D.wt
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Treatments


Seed reducing sugars
mg g-1 D.wt
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Treatments


Seed Sucrose
mg g-1 D.wt
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Treatments

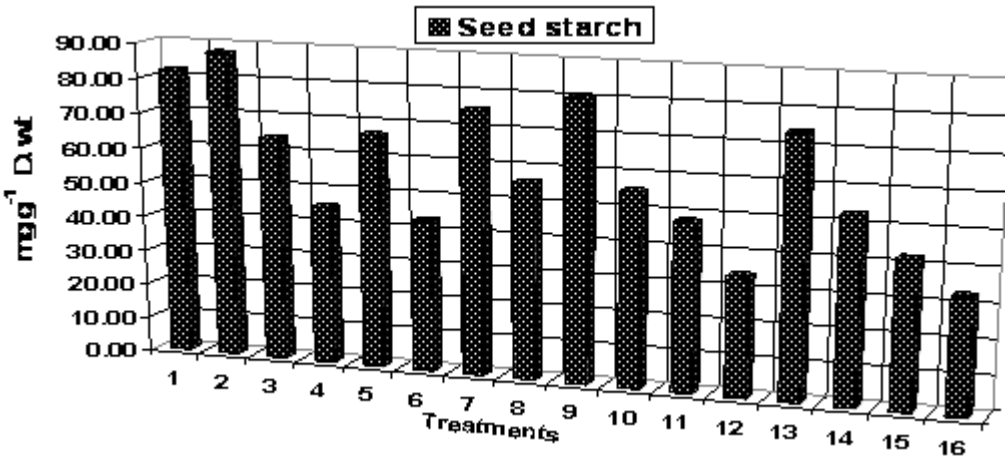


Figure 2: Biochemical analysis of *Vicia faba* seeds at 120-day old as affected by SeO_2 (0.4, 2.6 µg/ml), $HgCl_2$ and $CdCl_2$ (50, 200 µg/ml), and their mixtures. 1: Initial seed, 2: Control, 3: Hg 50 µg ml^{-1}., 4: Hg 200 µg ml^{-1}, 5: Cd 50 µg ml^{-1}, 6: Cd 200 µg ml^{-1}, 7: Se 0.4 µg ml^{-1}, 8: Se 2.6 µg ml^{-1}, 9: Hg 50 Se 0.4, 10: Hg 200 Se 0.4, 11: Hg 50 Se 2.6, 12: Hg 200 Se 2.6, 13: Cd 50 Se 0.4, 14: Cd 200 Se 0.4, 15: Cd 50 Se 2.6, 16: Cd 200 Se 2.6

Ref. [28] discussed that the interaction between Hg and plant systems is very important because Hg has largely been employed in seed disinfectants, in fertilizers and in herbicides.

With respect to influence of cadmium chloride, Ref. [29] decided that the elevation of soil Cd content not only causes toxic damage to the organelles and molecules of plants, finally leading to reduce yields. Ref. [30] stated that cadmium and nickel markedly reduced soybean plant biomass and seed production. Cadmium reduced mature seed mass. This effect was mostly due to decrease yields of lipids, protein and carbohydrates. Previously, Ref. [31] reported that lead (300 µM) and cadmium (18 µM) inhibit pod fresh weight in soybean (*Glycine max*) by 35%. The reduction of pod fresh weight correlated with the effect of lead and cadmium on several other aspects of plant metabolism (shoot, root, leaf, and nodule dry weight; nodule ammonia, protein and carbohydrate contents). Moreover, Ref. [32,33] stated that when the soils were contaminated with Zn and Cd, may be observed to a significant decrease in the amount of harvest which a serious problem for agricultural economies. Ref. [34] found that there was a significantly decrease in the weight gain depending on dose in *Phaseolus vulgaris* seeds exposed to 30 and 70 ppm of Zn and Cd ions when compared to control. Also, Cd treated seeds showed a lower weight gain than Zn treated seeds. So that Ref. [35] reported that heavy metal ions may block the entry of cations and anions into plant tissues. They also determined that heavy metals may cause a decline in transpiration rate and water content of plant tissues. These conditions may cause significant alterations in nutrient contents of tissues, and may reduce the weight gain and growth.

Ref. [36] studied the effect of cadmium toxicity (0, 0.1, 1 and 10 µg) in lint yield and yield component of three cotton genotypes (Zhongmian 16, Zhongmian 16-2 and Simian 3). They showed that lint yield of Simian 3 decreased at 0.1 and 1 µM Cd by 0.29 g and 2.21 g, respectively, compared to the control. However, the lint yield of Zhongmian 16 and Zhongmian 16-2 exposed to 0.1 µM Cd increased 3.68 g and 3.31 g in comparison to the control. However, at 1 µM Cd a decrease of 0.51 µg and 2.06 g was observed, respectively, when compared to control. The cotton plants exposed to 10 µM Cd grew too small to produce bolls. As to boll number per plant, an increase in Zhongmian 16 and Zhongmian 16-2 exposed to 0.1 µM Cd was observed by 0.66 and 1.33 g respectively, while a decrease of 0.34 was recorded in Simian 3, when compared to the controls. In the treatment with 1 µM Cd, a sharp decrease was found in three genotypes, and Simian 3 was most affected.

With respect to the effect of selenium dioxide on plant production, Ref. [37,38] showed non-detrimental effect of selenite on ryegrass yield at low Se addition levels. While, Ref. [39] showed that, based on the flowering stage of *Brassica napus* plants displayed reduced fruiting rates compared to those in the control. Within 45 days after formation of the first fruit, nearly all of the control plants showed evidence of fruit set. However, less than one-fourth of the selenium-treated plants had fruit in the same time. Most of the selenium-treated *Brassica napus* plants never did produce fruits; those that did formed more fruits per plant than plants in the control. There was no significant difference in number of seeds per fruit formed in the control and selenium-treated plants.

Ref. [40] discussed that it is plausible that low nitrogen levels and selenoproteins might both have the same effect on fruit set by effectively reducing the levels of functional proteins. This could be because selenium and sulfur are similar in structure and the plant often takes up selenium in the sulfur uptake pathways. This leads to competition between selenium and sulfur, which may result in inhibition of disulfide bonds in proteins, amino acids such as cysteine, co-enzymes sulfur is a part of, and metallothions. Disruption of metallothions would make the plant unable to deal with high levels of other metals that may be present in the tissue of the plant. Ref. [41] stated that a higher Se dosage (1.0 mg kg^{-1} soil) was toxic to lettuce and reduced the yield of young plants. In the senescing plants, it diminished the dry weight yield but not the fresh weight yield. Also, Ref. [42] reported that the yields of lettuce plant drop drastically at Se contents above 20 mg kg^{-1} dry weight. While, Ref. [43] indicated a slightly negative effect of Se on primary branching and on seed production in buckwheat. In contrast, Ref. [44,45] showed that the foliar application of selenium had significant and additive effects on canola plant number of pods per plant, number of seeds per pod, seed yield, biological yield and harvest index. Also, Ref. [46] stated that retention of move leaf area, with better partitioning efficiency, appeared to increase 100 seeds weight and total yield/plant in *Vicia faba* plants treated with selenium. Pretreatment of faba bean seed with selenium at 50 mg/L may contribute to delayed leaf senescence and improved total yield. Ref. [47] found that the addition of Se affected potato yield and average tuber size. Significantly higher yields were harvested from the Se-treated plants 15 weeks after planting. Also, Ref. [41] discussed that enhancement of photosynthesis and a decrease in leaf senescence by Se application increases assimilate production and transport towards seeds and as a result seed yield increases.

Furthermore, Ref. [48] indicated that Se is a beneficial trace element in potato plants that exerts a positive effect on yield formation and improves the processing and storage quality of potato tubers. These positive effects of Se are, however, dependent on the Se concentration and the age of the potato plant and tuber.

Ref. [49] reported that foliar Se fertilization of garlic at rates of 10-50 µg of Se/ml can be recommended to increase the number of large bulbs.

Results of the present study showed that applying selenium dioxide was highly significant attenuated the severe drop in all production criteria, which more pronounced at its lower than higher levels as compared to individual heavy metal salts. Concerning, the seed protein content was increased with applying both selenium dioxide levels but, the adverse effect was observed with reducing sugar contents. Addition low selenium dioxide to stressed plants increased both sucrose and starch contents to a great extent which more appeared at its lower than higher levels. Ref. [50] concluded that during heavy metal stress Se might prevent their toxic effect in sunflower plants. It has been suggested that the protective effects of Se are due to the formation of non-toxic Se metal complex [51].

Ref. [52] observed that the proportion of α-tocopherol was similar in the control of *Brassica oleracea* plants and those supplied with Se separately or in combination with cadmium. However the percentage of α-tocopherol concentration increased to the level of control and Se-enriched plants when Se was added simultaneously with Cd. It has been reported that an increase of α-tocopherol favors the stress tolerance of plants as it favors the scavenging of singlet oxygen species in chloroplasts [53,54]. Therefore, the increase of α-tocopherol in plants exposed to Se and Cd simultaneously, in comparison to those grown only in Cd, shows evidence that Se assists the plants in the adaptation.

According to the effect of heavy metal salts on protein profile, it is found that, expression of stress proteins in plants are known to be an important adaptive strategy of environmental stress tolerance. They are highly water soluble and heat stable, associated to cytoplasmic membranes and organelles and act as molecular chaperones [55,56].

In the present study, it could be demonstrated from Table 1 and plate 1 that the stress-protein expression was induced in the harvest seeds of *Vicia faba* exposed to $HgCl_2$, $CdCl_2$, SeO_2 or untreated plants. The electrophoretic pattern of all treatments showed three distinct regions depending upon the protein molecular weight, i.e., high proteins (>100 KDa), medium proteins (<60 KDa) and low molecular weight proteins (<20 KDa). Some protein bands were newly synthesized and some others were disappeared as it was appeared the synthesis of new bands at molecular weight 153 KDa of the seeds produced from treated plants by single heavy metal salts as compared to control and initial seeds. While, the seeds produced from untreated plants characterized by the appearance of new bands at molecular weights 39 and 37 KDa. Also, $HgCl_2$ was induced the appearance of new bands at molecular weights 106, 101 and 31 KDa. In the meantime, $CdCl_2$ caused appearance of new bands at molecular weights 100 and 96 KDa, while Se caused the synthesis of new bands at molecular weights 126, 122, 88 and 70 KDa. Combination of Hg Cl_2 with Se O_2 appeared new bands at molecular weights 79, 77, 71, 67 and 48 KDa, and new bands were detected at molecular weights 119, 108, 94, 92, 86, 81 and 62 KDa at combination of $CdCl_2$ with SeO_2.

Table 1: Relative concentration of proteins extracted from *Vicia faba* seeds, cv. Sakha 1 as affected by SeO_2 (0.4, 2.6 µg/ml), $HgCl_2$ and $CdCl_2$ (50, 200 µg/ml), and their mixtures. 1: Initial seed, 2: Control, 3: Hg 50 µg ml^{-1}., 4: Hg 200 µg ml^{-1}, 5: Cd 50 µg ml^{-1}, 6: Cd 200 µg, ml^{-1}, 7: Se 0.4 µg ml^{-1}, 8: Se 2.6 µg ml^{-1}, 9: Hg 50 Se 0.4, 10: Hg 200 Se 0.4, 11: Hg 50 Se 2.6, 12: Hg 200 Se 2.6, 13: Cd 50 Se 0.4, 14: Cd 200 Se 0.4, 15: Cd 50 Se 2.6, 16: Cd 200 Se 2.6.

KDa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
153	6.73	7.18	8.79	12.46	7.30	7.75	6.11	8.48	-	-	-	-	-	-	-	-
133	3.72	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
126	-	-	-	-	-	-	1.19	-	-	-	-	-	-	-	-	-
122	-	-	-	-	-	-	-	3.86	-	-	-	-	-	-	-	-
121	-	-	-	3.37	-	1.42	0.81	-	-	-	-	-	-	-	-	0.66
119	-	-	-	-	-	-	-	-	-	-	-	-	-	0.94	-	-
114	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.16	1.12
113	-	-	-	-	-	-	-	-	-	-	0.42	1.31	1.21	-	-	-
111	-	-	-	-	-	2.47	-	3.07	-	-	-	-	-	-	-	1.54
110	-	-	5.58	-	4.34	-	3.23	-	2.30	-	-	-	-	-	1.46	-
109	10.77	-	-	-	-	-	-	-	-	1.47	1.25	-	-	-	-	-
108	-	-	-	-	-	-	-	-	-	-	-	-	-	3.00	-	-
106	-	-	-	6.08	-	-	-	-	-	-	-	-	-	-	-	-
102	-	-	-	-	-	-	-	5.44	2.16	-	-	-	-	-	-	3.22
101	-	-	5.54	-	-	-	-	-	-	-	-	-	-	-	-	-
100	-	-	-	-	4.46	-	-	-	-	-	-	-	-	-	-	-
97	-	-	-	-	-	-	5.25	-	-	2.18	-	-	-	-	-	-
96	-	-	-	-	-	5.40	-	-	-	-	-	-	-	-	-	-
95	1.19	-	-	-	-	-	-	-	2.53	-	-	-	-	-	-	-
94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.04
93	-	-	-	-	-	-	-	-	-	0.99	1.85	-	-	-	6.42	-
92	-	-	-	-	-	-	-	-	-	-	-	-	-	5.04	-	-
90	-	-	-	-	-	2.66	-	-	-	0.82	-	-	-	-	-	0.91
88	-	-	-	-	-	-	-	3.26	-	-	-	-	-	-	-	-
87	-	9.30	-	-	-	-	4.10	-	-	-	-	-	-	-	-	1.95
86	-	-	-	-	-	-	-	-	-	-	-	-	-	2.49	-	-
85	-	-	3.11	7.46	2.72	-	-	-	3.61	-	-	-	-	-	1.21	-
84	7.51	-	-	-	-	-	-	-	-	2.17	-	5.97	-	-	-	-
83	-	-	-	-	-	-	-	-	-	-	2.56	-	9.80	-	-	-
82	-	-	-	-	-	-	-	-	-	-	-	-	-	1.81	1.32	-
81	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.11
80	-	3.32	-	9.28	-	-	-	-	-	-	-	-	-	-	-	-
79	-	-	-	-	-	-	-	-	2.13	-	-	-	-	-	-	
KDa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
77	-	-	-	-	-	-	-	-	-	-	-	4.18	-	-	-	-
76	-	-	-	-	-	-	-	-	-	2.03	-	-	5.42	-	1.96	-
75	-	-	-	-	-	-	-	-	-	-	1.68	-	-	1.90	-	-
73	-	20.11	5.56	-	-	9.24	-	-	-	-	-	-	-	-	-	-
72	7.27	-	-	-	3.82	-	-	9.37	-	2.81	-	-	-	-	-	-
71	-	-	-	-	-	-	-	-	3.22	-	-	-	-	-	-	-
70	-	-	-	-	-	-	4.88	-	-	-	-	-	-	-	-	-
69	-	-	-	-	-	-	-	-	-		2.19	-	-	-	-	4.45
68	-	-	-	-	-	-	-	-	-	-	-	-	-	3.20	2.66	-
67	-	-	-	-	-	-	-	-	2.96	-	-	-	-	-	-	-
66	-	-	4.76	-	-	-	3.34	-	-	-	-	-	-	-	-	-
65	-	-	-	-	3.30	-	-	3.46	-	-	-	-	-	-	2.57	-
64	-	-	-	3.60	-	-	-	-	-	-	1.62	-	-	3.53	-	3.27
63	-	-	-	-	-	-			1.99	3.89	1.65	-	-	-	-	-
62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.66	-
61	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-
60	-	2.94	10.58	4.96	5.20	4.52	6.43	-	7.73	6.58	-	7.78	-	-	-	-

59	-	-	-	-	-	-	-	9.02	-	-	3.86	-	-	5.58	5.33	5.27
58	15.24	5.49	-	5.53	-	-	4.06	-	-	-	-	2.19	9.13	-	-	-
57	-	-	-	-	-	-	-	-	-	-	2.94	-	1.56	-	-	-
56	-	-	-	-	-	-	1.80	-	-	1.65	-	-	-	-	-	-
55	-	-	-	-	-	-	-	-	-	-	1.57	-	-	-	1.24	-
54	-	-	-	2.89	-	-	-	-	-	2.34	-	-	-	-	-	-
53	-	4.27	1.79	-	3.78	-	4.07	-	-	-	2.55	5.91	-	4.28	2.42	-
52	-	-	-	-	-	5.09	-	-	7.13	4.39	-	-	-	-	-	5.93
51	-	3.46	5.02	-	3.91		4.33	-	-	-	4.59	-	10.35	4.36	4.40	4.57
50	4.56	-	-	3.75	-	9.15	-	5.02	-	-	-	4.15	-	-	-	-
48	-	-	-	-	-	-	-	-	2.31	-	-	-	-	-	-	-
47	-	-	-	-	-	-	1.91	-	-	2.94	3.44	-	-	-	-	-
46	-	-	1.56	-	1.26	-	-	-	-	-	-	-	-	10.65	2.90	-
45	-	8.27	-	-	-	5.43	-	5.17	-	-	-	-	18.08	-	-	2.42
44	-	-	-	-	-	1.39	-	-	-	-	-	-	-	-	-	4.03
43	-	-	-	-	11.23	-	-	-	-	9.25	-	-	-	-	-	-
42	17.84	-	7.80	-	-	1.84	7.29	-	-	-	14.35	14.90	-	-	13.75	3.01
41	-	-	-	-	-	-	-	10.90	19.27	-	-	-	-	-	-	5.45
40	-	-	7.83	4.10	3.57	10.71	3.53	-	-	4.02	-	-	-	8.81	-	-
39	-	2.71	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KDa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
38	-	-	-	5.08	3.01	-	5.80	-	-	6.77	7.87	13.05	5.18	-	3.11	3.81
38	-	-	-	5.08	3.01	-	5.80	-	-	6.77	7.87	13.05	5.18	-	3.11	3.81
37	-	3.57	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	-	-	2.12	-	-	-	-	-	-	-	-	-	-	1.83	2.61	1.96
35	-	-	-	-	2.75	2.29	-	-	-	-	-	-	--	2.21		2.59
34	-	-	-	-	-	-	-	3.39	-	-	3.44	3.53	11.63	-	-	-
33	-	-	7.45	4.19	5.77		7.33		8.41	9.24	5.95	2.10	-	-	-	-
32	7.23	-	-	2.60	-	6.87	-	4.98	-	-	-	3.48	-	7.62	10.22	6.66
31	-	-	1.20	-	-	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	4.06	0.40	-	-	2.99	-	-	-	-	-	3.28	-	-
29	4.64	15.53	4.48	5.08	-	-	5.46	-	-	12.12	-	12.63	10.79	-	9.74	-
28	-	-	-	-	2.89	5.94	5.89	-	-	-	12.70	-	-	-	-	-
27	-	-	3.16	-	5.03	-	1.15	4.31	-	-	-	-	-	-	-	-
26	-	-	-	-	15.35	-	-	-	-	-	-	-	-	7.64	-	-
25	5.54	1.97	5.66	2.15		8.17	4.82	7.65	17.05	7.03	2.79	2.37	2.35	8.47	9.07	16.80
24	3.39	2.78	-	1.34	1.74	-	-	-	-	-	4.67	3.68	1.36	-	-	-
23	-	-	-	-	-	-	-	-	7.71	-	-	-	-	-	-	8.85
22	-	-	-	-	3.79	4.02	3.19	2.43	-	7.33	-	-	3.89	-	-	-
21	-	2.24	3.03	-	-	-	0.88	-	-	-	3.67	2.93	-	-	-	-
20	2.62	-	-	-	-	1.98	1.13	2.44	-	-	2.84	-	0.85	3.40	6.39	-
19	-	0.20	-	-	1.32	-	2.06	1.57	3.42	4.27	3.81	-	-	2.56	1.48	1.35
18	-	2.02	1.92	8.54	1.64	1.45	-	2.05	-	-	-	-	-	1.45	1.65	2.28
17	-	4.64	2.23	2.18	0.79	1.55	2.99	-	5.58	4.15	-	-	-	-	4.15	2.88
16	1.76	-	0.83	1.31	0.65	0.65	0.98	1.13	-	1.56	5.77	9.85	8.32	4.95	1.11	1.21
15	-	-	-	-	-	-	-	-	0.51	-	-	-	-	-	-	0.18
Total number of bands	15	17	22	21	25	22	28	21	18	23	25	17	15	25	25	28

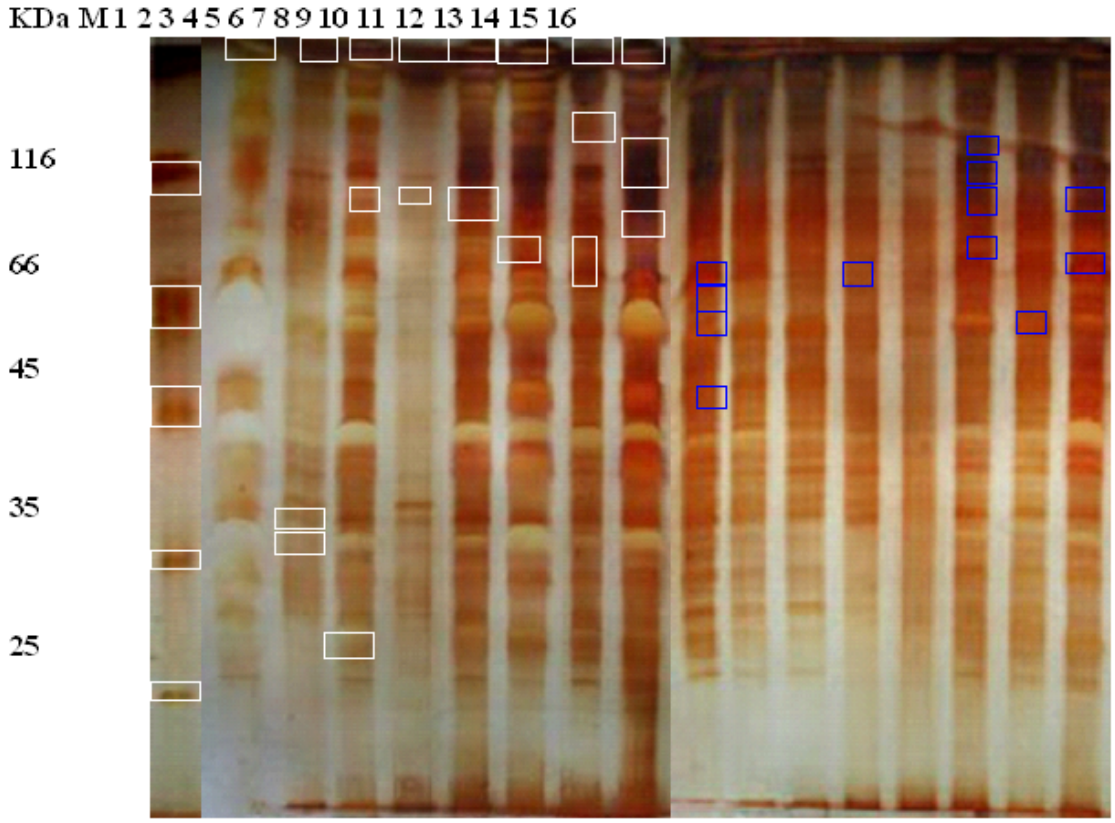


Plate (I): One-dimensional SDS-PAGE of proteins extracted from seeds of 120-day Old *Vicia faba*, Sakha 1 as affected by SeO_2 (0.4, 2.6 µg/ml), $HgCl_2$ and $CdCl_2$ (50, 200 µg/ml), and their mixtures. 1: Initial seed, 2: Control, 3: Hg 50 µg ml^{-1}., 4: Hg 200 µg ml^{-1}, 5: Cd 50 µg ml^{-1}, 6: Cd 200 µg ml^{-1}, 7: Se 0.4 µg ml^{-1}, 8: Se 2.6 µg ml^{-1}, 9: Hg 50 Se 0.4, 10: Hg 200 Se 0.4, 11: Hg 50 Se 2.6, 12: Hg 200 Se 2.6, 13: Cd 50 Se 0.4, 14: Cd 200 Se 0.4, 15: Cd 50 Se 2.6, 16: Cd 200 Se 2.6.

Concerning, presence of new protein bands at molecular weight 15 KDa particularly with seeds produced from plants treated with Hg Cl_2 50 µg ml^{-1} combined with SeO_2 0.4 µg ml^{-1} and $CdCl_2$ 200 µg ml^{-1} combined with SeO_2 2.6 µg ml^{-1}. These results were in conformity with Ref. [57] found that both Pb and Hg induced changes in the SDS-gel electrophoretic patterns as mercury treatment disappeared the bands of 79 and 14.2 KDa at 1.0 µM in *Hydrilla verticillata*, however, two new bands of 20 and 21 KDa appeared at the same concentration in the roots of *Vallisneria spiralis*, this may have implication in metal tolerance and also, the reduction or disappearance of bands could be due to the interference of metal ions with the *de novo* protein synthesis, also he reported that the plants when exposed to metal stress conditions induced the synthesis of new protein while the synthesis of cellular protein ceased. Ref. [58] have reported that protein profiles were altered at heavy metal stress condition.

Ref. [59] found that there have been several reports of an increase in heat shock protein (HSP) expression of plants in response to heavy metal stress. Ref. [60] showed that, in rice, both heat and heavy metal stresses increased the levels of mRNA for low molecular weight heat shock proteins (16-20 KDa). Small heat shock proteins (e.g., HSP 17) were also shown to be increased in cell culture of *Silene vulgaris* and *Lycopersicon peruvitoanum* in response to a range of heavy metal treatments [59].

Ref. [61] reported that the protein with an apparent molecular weight of 12 KDa, found in pea roots treated with 0.05 mM Cd might be a putative phytochelatin. Formation of this new protein would be part of the significant increase in total protein concentration in pea root. Moreover, after dialysis, most of the Cd remained associated with material of higher molecular weight than 68 KDa which supports the association of Cd with polypeptides. Ref. [62] who suggested that up to 85% of Cd were bound to proteins of low molecular weight in roots of maize. Ref. [63] discussed that the disappearance of polypeptide bands may be due to the precipitation of these proteins, or their use as a precursor for some antioxidant molecules in conjunction with protein.

Ref. [64] revealed that a large scale disruption of protein banding patterns and banding intensity by $CdCl_2$ treatment (0, 20, 40, 80, 160 ppm) resulting in an appreciable decrease in the number of protein bands in *Vigna unguiculata* shoots and roots over those of the untreated and this may be attributed to the metal induced inhibition of protein synthesis. Ref. [65] reported that since protein synthesis is ATP dependent, stress induced a decrease in energy charge which probably contributed to the decrease in protein synthesis.

Several studies have pointed out that Cd interferes with the uptake, transport and distribution of essential mineral nutrients in plants [66], which may directly or indirectly affect protein synthesis. The most important metal ion in this respect was Mg which is an essential cofactor for the activity of many enzymes involved in transcription and translation [67]. High doses of Cd may compete with Mg binding to the enzymes [68], and hence, lower activity of the enzymes involved in DNA, RNA and protein synthesis. Thus, the reduction in soluble protein level with the corresponding decrease in protein banding patterns and intensity could be attributed to Cd stimulating hydrolytic enzyme activities. Ref. [69] showed a decline in protein and RNA levels with a corresponding increase in the activity of hydrolytic enzymes such as protease and ribonuclease. Stimulation of ribonuclease in Vigna unguiculata shoots and roots was reported by Ref. [70], and this paralleled with the decrease in protein levels.

Ref. [71] found that quantitative and qualitative differences were observed in polypeptide patterns. Ten polypeptide bands are detected in the green tops of control *Eruca sativa* plants but, 11 polypeptide bands are shown in Se-treated leaves. Moreover, a new polypeptide with molecular weight 9.2 KDa is detected in the green *Eruca sativa* tops exposed to selenate. These new polypeptides may be related to metal binding polypeptides phytochelations (cadystins). Ref. [72] who stated that selenate anions induced the synthesis of phytochelatins (3-10 KDa). Ref. [73] proposed that plant cells synthesize organic acids or polypeptides that chelate metal ions, which is required for some tolerance mechanisms. However, Ref. [74] reported that the substitution of selenium for sulfur alters the redox properties of these proteins.

In the present results, there were no bands synthesized at the treatments $HgCl_2$ 200 μg ml^{-1} combined with SeO_2 0.4 μg ml^{-1}, $HgCl_2$ 50 μg ml^{-1} combined with SeO_2 2.6 μg ml^{-1} and $CdCl_2$ 50 μg ml^{-1} combined with SeO_2 0.4 μg ml^{-1} as compared to control and initial seeds. This may be attributing to the formation of Hg-Se and Cd-Se complex compounds which were insoluble and immobile compounds cannot be translocated through root plasma membrane into the *Vicia faba* plant.

Ref. [48] reported that in higher plants, metabolism of Se is closely related to that of sulfur due to their chemical similarity. The non-specific incorporation of the selenoamino acids (selenomethionine and selenocysteine) into proteins is thought to be the major cause of Se toxicity in non- accumulator plants supplied with a high Se dose [11]. Ref. [75] suggested that one explanation for higher toxicity of selenite compared to selenate is that after uptake selenite is incorporated faster than selenate into selenoamino acids in roots. High Se concentrations were shown to provoke oxidative stress response such as increased lipid peroxidation in plants [76].

Ref. [77] explained that the plant can convert Se mainly into Se-methionine (Se-Met) and incorporate into protein in the place of methionine which may account for >50% of the total Se content of the plant. Moreover, Ref. [78] reported that the organisms that require Se for normal function, development and reproduction produce a wide array of seleno-proteins which contain one or more Se-cystine (Se-Cys) residues integrated into the main polypeptide backbone via a unique co-translational process. Such selenoproteins include glutathione peroxidase, formate dehydrogenase and selenophosphate synthetase. Ref. [79] stated that high molecular-weight selenium/mercury-containing compounds were found primarily in the *Brassica juncea* root extract. Evidence suggests that a Se complex of high molecular weight may be protein associated.

According to Ref. [80], the presumed protective effect of Se against cadmium and mercury toxicity is through the diversion in their binding from low-molecular weight proteins to higher-molecular weight ones.

CONCLUSION

It could be presumed that expansion of selenium may be lightening the lethal impact of mercury and cadmium on *Vicia faba* production. So that it could be used selenium to alleviate the stress of heavy metals.

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