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Interaction between Sulfur and A-Tocopherol and Their Effects on Alleviating Selenium Toxicity in Mung Bean Cuttings and Seedlings

Abdullah I. Shaheed Rihab E. Kadhim* Ahmed J. Abdul-Kadhim
Univ. of Babylon, College of Science, Dept. of Biology, P.O. Box (4) Hilla, Iraq

Abstract
Physiologically, the sulphur (S) and α-tocopherol (α-T) role in alleviating selenium (Se) toxicity has been studied by using Hoagland solution as balanced environment for S and Se content in mung bean cuttings & seedlings. However, toxic selenium level at optimum promontory concentrations of both S and α-T were supplied to seedlings and cuttings of mung bean as experimental system, which taken from 10-day-old light grown seedlings under standard conditions.

The results indicated the following:

1. Raising of Se level in cutting and seedling parts (roots, hypocotyls, epicotyl and leaves) significantly, when Se was supplied individually.
2. Significant decline in Se-uptake and accumulation in plant tissues ,when supplied with S whereas, α-T had no effect on uptake that encouraging its translocation & accumulation in leaves .There after it causes , diminishing its conc. in hypocotyls and epicotyls . Consequently, Se level was declined in hypocotyls in both cases when S and α-T supplied individually whereas, the situation was different when supplied together with Se , in terms of S & Se content.
3. The mechanism of Se-tolerance in seedlings of mung bean is reside in roots, that causes its accumulation and sequestration in seedling roots at high level coincided with low amount acropetally translocated, leaves (in case of using mung bean seedlings instead of cuttings).

Keywords: detoxification, mung bean cuttings, rooting response, selenium, sulfur, and α- tocopherol.

1. Introduction

Selenium was considered as one of the beneficial elements. Chemically is similar to sulphur and tellurium (Te). Selenium displays metalloid characteristics and it is found in several different oxidative forms such as elemental selenium (Se0), selenide (Se2−), selenite (Se4+), and selenate (Se6+9). Martens and Suarez (1999) mentioned that Se-C bonds can also formed directly, and it is found in a variety of organic compounds such as methylated compounds, selenoamino acids, and selenoproteins. Selenite (SeO4) is the most abundant bioavailable form of Se in soils, plants take it via sulfate transporters in roots (Shibagaki *et al.*, 2002; Ellis and Salt, 2003). However, after uptake, selenate is thought to be transported into chloroplasts of leaves. Leustek (2002) indicated that indistinguishable to most S enzymes, Se can be found in most S-containing metabolites. The S assimilation pathway first reduces selenate to selenite, in chloroplasts, then to selenide, which is incorporated into selenocysteine (SeCys) and selenomethionine (SeMet) enzymatically (Terry *et al*., 2000; Ellis and Salt, 2003; Sors *et al.*, 2005). Stadtman (1996) mention these two seleno-amino acids can be with mistake incorporated into proteins, replacing Cys and Met, which causes Se toxicity. Sulfur transporters are different in their affinity to S & selenite (White *et al*., 2004); in addition to that Se-assimilation was following S-assimilation pathways and involving the same enzymes & the same binding sites as mentioned by Terry *et al.* (2000) & Sors *et al*. (2005).

Interestingly, S may affect Se –absorption through two physiological processes in plants: 1) Sulfur competes Se on membrane transporters. 2) Sulfur affects Se by regulating S-transporters, and S-transporters were regulated by internal S-status. The expected strong effect of S on Se revealed only when plants under S-shortage (Li *et al*., 2008; Stroud *et al*., 2010). In addition to the competition between S & Se in vital process such as absorption ,transport and digestion through plant development (Rausch & Wachter, 2005).Consequently, S affect Se-accumulation in plants by acting on diminishing of Se-absorption in alfalfa (Mikkelsen, 1989).

Recently, it has been found that in terms of adventitious root formation (ARF) of mung bean cuttings was reduced ≈ 50% at 1.5 µM/L of Se (toxic level) (Shaheed *et al*., 2013). The latter showed that detoxification of Se was occurred by employing of S and α-T individually or in combinations.

2. Materials and Methods

Seeds of mung bean (*Phaseolus aureus* Roxb.) were soaked for overnight in current water, germinated in sterilized sawdust under standard conditions. The latter involved continuous illumination, light intensity of 1600-1800 lux, temperature of 25 ± 1°C and R.H of 60-70% in growth chamber (Binder KBW plants growth chamber).

Hoagland solution (half strength) in particular solution no. 2 that deals with macro-elements was used (according to Hoagland & Arnon, 1950) in solution culture for grown stock plants.

Cuttings were prepared from 10-day-old light grown seedlings according to (Hess, 1961) as experimental system. Such cuttings consist of small apical bud, pair of fully expanded primary leaves, epicotyl and hypocotyl (3 cm length) under cotyledonary nodes, after removal of root system.

Tested solutions were involved in the current study are: boric acid at (5 µg/ml) as a rooting medium for the essential boron role in growth & root primordia development into visible roots (Middleton, 1978 & Shaheed *et al.*, 2010).

However, Se was prepared as selenite (Na_2SeO_3) at 1.5 µM/L (toxic level in terms of ARF), in addition to sulfur as $MgSO_4.7H_2O$ at 2 mg/L and α-Tocopherol at 0.1 µM/L (optimal concentrations in terms of ARF). Selenium was measured according to (APHA, 1985) using, atomic absorption at 335 nm and hydrate index. Pure Se was used at different concentration for standard curve. Sulfur measured according to (APHA, 1985).

3. Results

Table 1 shows the S and α-T effect in Se-toxicity in uptake & distribution of toxic Se in cutting parts. However, the results were revealed that Se conc. in hypocotyls, epicotyls & leaves of control cuttings 0.029, 0.0187 and 0.0195µg/g respectively. Consequently, Se-content was increased significantly in cutting after application of toxic-Se to 0.19, 0.28 and 0.198µg/g in the above parts respectively. For controlling Se-toxicity and its distribution, cuttings were supplied with combination (Se + S), (Se + α-T) and (Se + α-T + S) that caused significant decline in Se conc. in all cutting parts particularly at combination (Se + S). Accordingly ,S was caused detoxification of Se not only in cutting parts as individual but in terms of se-content in the whole cutting too(≈ 40%) compared to Se treatment.

Table 1: Effect the sulfur & α-tocopherol in uptake and distribution the selenium toxicity in different parts of mung bean cuttings.

Treatment	Selenium(µg/g)			
	hypocotyls	Epicotyls	leaves	Whole cutting
Hoagland (H)	0.029	0.0187	0.0195	0.0672
Selenium(Se)	0.19	0.28	0.198	0.668
Tocopherol(α-T) α-	0.02	0.044	0.018	0.0821
Sulfur (S)	0.01	0.0104	0.012	0.0324
T α-Se +	0.15	0.21	0.242	0.602
Se + S	0.108	0.154	0.145	0.407
Se + S + α-To	0.12	0.14	0.171	0.431
L.S.D	0.027	0.058	0.037	0.121

Table 2 shows that Se-conc.in roots, hypocotyls, epicotyls and leaves of seedlings of control treatment (Hoagland solution) were 0.082, 0.035, 0.045 and 0.057µg/g respectively. These figures increased significantly in seedlings after toxic-Se application to 0.487, 0.216, 0.24, 0.247µg/g in the above parts respectively. Obviously, the higher conc. of Se was located in roots. In other words, roots were sequestrates 41.5% of total Se. For controlling Se-toxicity & distribution of toxic Se in plant tissues, seedlings were supplied with different combinations (Se + S, Se + α-T and Se + α-T + S) that caused:

a) A decline in Se conc. in seedling parts, such decline was significant particularly with combination (Se + S) in roots only , that reflects a signification decline too in terms of a whole seedling compared to control (Se).

b) No significant decline with the combination (Se + α-T) in all of the above parts of seedling as well as in terms of the whole seedling compared to Se-treatment except the significant increase in leaves.

c) No significant decline with the combination (Se + S + α-T) in all of the above parts of seedlings except the significant decline in terms of the whole seedling. Seemingly, the presence of S in the last combination caused diminishing the uptake of total content of Se in terms of the whole seedling as well as in all parts of seedling. Whereas , α-T have no effect on the total content (uptake) but influence the transport of Se toward the leave, that enhances accumulation of Se in leaves in comparison to its diminishing in roots, hypocotyls and epicotyls.

Table 2: Effect the sulfur & α-tocopherol in uptake and distribution the selenium toxicity in different parts of mung bean seedlings.

Treatment	Selenium(µg/g)				
	Root	Hypocotyls	Epicotyls	leaves	Whole cutting
Hoagland (H)	0.082	0.035	0.045	0.057	0.219
Selenium (Se)	0.487	0.216	0.24	0.247	1.19
Tocopherol(α-T)α-	0.093	0.028	0.054	0.02	0.195
Sulfur(S)	0.086	0.024	0.09	0.024	0.224
TαSe +	0.425	0.183	0.29	0.342	1.24
Se + S	0.25	0.125	0.175	0.214	0.764
Se + S + α-To	0.418	0.154	0.17	0.185	0.927
L.S.D	0.12	0.093	0.1	0.075	0.186

On the other hand, the uptake and distribution of sulfur in different parts of cuttings were shown in table 3. The latter revealed that S-conc. in hypocotyl, epicotyl and leaves of control cuttings was 0.096, 0.134 and 0.457mg/g respectively. However, S-content was increased in cuttings were treated with S at 2mg/L to 0.1125, 1.79 and 0.521 mg/g in the above parts respectively compared to general control (Hoagland solution.) Although, such increase was not significant statistically on the 0.05 level of the probability .However, when Se supplied at toxic level that caused decreased in S-conc. to 0.0775, 0.102, 0.357mg/g in the above parts compared to control, and such decline was not significant. Although the increase (0.812 mg/g) or decrease (0.543 mg/g) in S-content after supplying S or Se respectively in terms of the whole cutting was not significant too.

In addition, when cuttings were supplied with different combinations, all caused decline in S-conc. in all parts of the cutting compared to that supplied with S-alone. Consequently, the decline was significant in leaves only and for all combinations, in addition to the epicotyl of cuttings supplied with the combination Se + α-T. Note withstanding, in terms of the whole cutting the decline was significant in all combination compared to S-treatment except the combination (Se + S + α-T) where the decline was not significant.

Table 3: The Effect of uptake and distribution of sulfur in selenium detoxification of different parts of mung bean cuttings.

Treatment	Sulfur(mg/g)			
	Hypocotyls	Epicotyls	leaves	Whole cutting
Hoagland(H)	0.096	0.134	0.457	0.687
Selenium(Se)	0.0775	0.109	0.357	0.543
Tocopherol(α-T)α-	0.095	0.111	0.415	0.621
Sulfur (S)	0.1125	0.179	0.521	0.812
Tα-Se+	0.075	0.104	0.334	0.513
Se + S	0.105	0.128	0.377	0.610
Se + S + α-To	0.0975	0.177	0.394	0.668
L.S.D	0.05	0.07	0.098	0.18

Uptake and distribution of sulfur in different parts of 10-day –old light grown seedlings has been shown in table (4). The latter revealed that S-conc.in roots, hypocotyl, epicotyl and leaves of control seedlings was (0.147, 0.163, 0.212 and 0.612) mg/g respectively .These levels were increased in seedlings supplied with S at 2 mg/L to (0.2, 0.184, 0.201, and 0.67) mg/g in the above parts respectively, compared to general control (Hoagland solution). However such increase is not significant in seedling parts, or in terms of the whole seedling too. Mean -while application of Se at toxic level reduces S-conc. to 0.106, 0.101, 0.175 and 0.457mg/g in the above parts compared to control except the decline was significant in leaves only. Moreover ,application of different combinations (Se + S),(Se+α-T and Se +S+ α-T) were caused significant reduction in S-conc. in seedling parts except the combination (Se+ α-T).The sulfur content at the last combination was 0.189mg/g compared to supplying S alone (0.201mg/g), in addition to significant decline in epicotyl & leaves only with the combination of Se + S. Whereas, the decline was significant at combination (Se + S + α-To) in roots & leaves only. Consequently, in terms of the whole seedling the decline was significant in all combination.

Table 4: Uptake and distribution of sulfur in selenium detoxification of different parts of mung bean seedlings.

Treatment	Sulfur(mg/g)				
	Root	Hypocotyls	Epicotyls	leaves	Whole cutting
Hoagland(H)	0.147	0.163	0.212	0.612	1.134
Selenium(Se)	0.106	0.101	0.175	0.457	0.839
Tocopherol(α-T)α-	0.127	0.154	0.189	0.444	0.914
Sulfur(S)	0.2	0.184	0.201	0.67	1.255
T α-Se+	0.088	0.108	0.189	0.447	0.832
Se+S	0.152	0.118	0.145	0.441	0.856
Se + S +α-T	0.113	0.130	0.165	0.422	0.83
L.S.D	0.078	0.067	0.054	0.112	0.37

4. Discussion

Exogenous application of selenium to Mung bean cuttings& seedlings (tables 1 and 2), was increased its concentration significantly in roots, stems and leaves. These results are in agreement with many studies such as (Xiao-Zhang *et al.,* 2008; Magdalena & Matgorzata, 2012; Schiavona *et al*., 2012). However, Se was taken up actively by roots (Terry *et al*., 2000) as show in table 2 and to half of this value in cuttings (table 1).Consequently, the physio-chemical similarities between Se &S makes their absorption by plant was closely similar. So the active absorption of sulfur by plant, mainly as SO_4 (Terry *et al.*, 2000). Mean - while the absorption of SO4 & SeO4 was as Leggett & Epstein (1956) mentioned. They referred that the absorption controlled by the same transporter with similar affinity for both ions.

For controlling Se-toxicity, it seems to be occurred via controlling its uptake & distribution through cuttings. The latter were supplied with different combinations such as (Se + S), (Se + α-T) and (Se + S + α-T) that caused significant decline in Se conc. in all cutting parts & by all combinations except the significant increase in leaves of cutting supplied with (Se+ α-T). Such trend reflects two cases from table 1 & 2 that deals with the amount of Se taken up and distributed through cutting parts. First, decline of Se content in hypocotyl and epicotyl and its increase in leaves with no change of Se conc. in cuttings supplied with (Se + α-T) in terms of its content in the whole cutting compared to cutting supplied with Se alone as control . This shows that α-T does not effects Se-uptake but enhance its transport to leaves. Second, the decline of Se –content in all cutting parts and for all treatment ,in addition to the decline (40%) in all treatment in terms of whole cutting compared to supplied Se alone as control . This shows that, application of Se alone or in combination between S+ α-T with Se, influence Se uptake in the first place rather than its decline in all cutting parts after its distribution. As a general conclusion, the sulfur or combination between (Se + S) was caused Se-detoxification for approximately 50% not only in cutting parts as individual but in terms of its content in the whole cutting too compared to Se (as control) thought the effect of S in preventing or diminishing Se-uptake. In addition, (Schiavona *et al*., 2012) was reported an antagonistic relation-ship in terms of absorption between S & Se as well as to the role of sulfur in alleviating Se conc. in *Brassica juncea* tissues. Consequently, (Li *et al.*, 2008) found that Se was highly accumulated in wheat roots when supplied as SeO_3 compared to SeO_4 because, SeO_4 was transported easier compared to SeO_3. The latter was in agreement with results of table 1. Whereas, tables 3 & 4 showed uptake & distribution of S in different parts of cutting or seedling respectively. Obviously, S supplied exogenously increase its conc. in leaves, stem and roots. SeO_4 was taken up actively, through plasma membrane of root cells (table 4),then transported to the shoot via transpiration stream (Davidian *et al.*, 2000).When absorbed by roots either accumulated in vacuoles of root or shoots or reduced in complex metabolic pathways. Notwithstanding the 1^{-st} steep involved absorption of S by root cells & reduced far from leaf chloroplast which considered the main location for S digestion (Davidian & Kopriva, 2010). These are in agreement with the current results represented in table 3 & 4. It was revealed that accumulation of S in high ratio in leaves compared to other parts of cutting .Thereafter, reduction of SO_4 to cysteine will change the oxidative number from (6^+) to (4^-) and that required to transport ten electrons. Whereas, GSH, ferredoxin, NADPH & *O*-acetylserine may acts as donors of electron in different pathway steps (Taize & Zeiger, 2003). Presumably, the photosynthesis supply reduced ferredoxin and photorespiration regenerate serine, both may enhances the production of *O*-ocetylserine. This state make leaves more active than roots in sulfur assimilation. It is noteworthy, that assimilated sulfur in leaves was exported via phloem to protein synthesis locations (root, shoot apex & fruits) that frequently form glutathione (Bergmann & Zeiger., 1993). However, GSH that synthesized from cysteine ,acts as signal to coordinate with the absorption of SO_4 by roots & finally assimilated in shoot (leaves).When Se supplied to cuttings & seedlings, that caused significant decline in S conc. in the whole cutting as well as in the whole seedling .It was attributed to the antagonistic role during absorption between S & Se. These results confirms the competition role of S with Se that reduce its average of absorption , hence, its distribution .In addition to the role of α-T in repairing the damage that occurred in cytoplasmic membrane , and finally diminishing Se absorption.

5. Conclusions

Mung bean is accumulator for Se element. S caused declination of Se uptake and accumulation in roots and all parts of mung bean, whereas α-T is represented as helper for Se transport and accumulates it in leaves. The competition role of S with Se reduced its absorption and its distribution. In a combination of Se + S + α-T, α-T caused diminishing of Se absorption.

References

APHA, AWWA, WPCF. (1985). Standard Methods for the Examination of Water and Wastewater. 16th Ed. Washington, D.C. APHA.

Arnon, D. R., & Hoagland, D. R. (1940). Crop production in artificial solutions & in soils with special reference to factors affecting yields & absorption of inorganic nutrients. Soil Sci., 50: 463-484.

Bergmann, L. & Zeiger, E. (1993).Glutathione metabolism in plants. In: DeKok, L. J.; Stulen, I.; Rennenberg, H.; Brunold, C. and Rauser, W.E. (eds.). Sulfur Nutrition and Assimilation in Higher Plants. Regulatory, Agriculture and Environmental Aspecets. SPB Academic Publishers, The Hague, Netherland, pp.102-123.

Davidian, J. C.; Hatzfeld, Y.; Cathala, N.; Tagmount, A. & Vidmar, J.J. (2000). Sulfate uptake and transport in plants. In: Brunold, C., Rennenberg, H., DeKok, L.J., Stulen, I., Davidian, J. C. (eds.). Sulfur Nutrition and Sulfur Assimilation in Higher Plants: Molecular, Biochemical and Physiological Aspects. Bern, Switzerland, Paul Haupt Verlag, pp. 19–40.

Davidian, J.C. & Kopriva, S. (2010). Regulation of sulphate uptake and assimilation the same or not the same? Molecular Plant, 3: 314–325.

Ellis, D.R. & Salt, D. E. (2003). Plants, selenium and human health. Curr. Opin. Plant Biol., 6: 273-279.

Hess, C.E. (1961). The mung bean bioassay for detection of root promoting substances. Plant Physiol. (suppl.): 37-21.

Hoagland, D.R. and Arnon, D.I. (1950). The water-culture method for growing plants without soil. California Agricultural Experiment Station Circular, 347:1-32.

Leggett, J.E. & Epstein, E. (1956). Kinetics of sulfate absorption by barley roots. Plant Physiol., 31:222 -226.

Leustek, T. (2002). Sulfate metabolism. In: C.R. Somerville, & E.M. Meyerowitz, (eds.), The *Arabidopsis* Book. American Society of Plant Biologists, Rockville, MD, USA.

Li, H.F., McGrath SP, & Zhao FJ (2008) Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. New Phytol., 178: 92- 102.

Magdalena,M-Z &Małgorzata,W. (2012). The influence of selenium on root growth and oxidative stress induced by lead in *Vicia faba* L. minor. Plants Biol. Trace Elem. Res., 147:320–328.

Martens, D.A. & Suarez, D.L. (1999). Selenium in water management wetlands in the semi-arid west. Hort. Sci., 34:34–39.

Middleton, W.; Javis, B. C & Booth, A. (1978). The boron requirement for root development in stem cuttings of *Phaseolus aureus* Roxb.. New Phytol., 81: 287- 297.

Mikkelsen, R.L. Haghnia, G.H. & Page. A.L. (1989). Effects of pH and selenium oxidation state on the selenium accumulation and yield of alfalfa. J. Plant Nutr., 10:937–950.

Schiavona, M. Pittarello, M.Pilon-Smits , E.A.H, Wirtzc, M. Hellc R. & Malagolia M(2012). Selenate and molybdate alter sulfate transport and assimilation in *Brassica juncea* L. Czern.: Implications for phytoremediation. Environ. & Exp. Bot., 75: 41– 51.

Shaheed, A. I. & Muhammad, A. J. (2010). The role of salicylic acid in alleviating boron toxicity in mung bean cuttings. Iraqi National J. Chem., 39:589-604.

Shaheed, A. I.; Kadhim, R. E. and Abdul-Kadhim A. J. (2013). The protective role of sulfur and α-tocopherol in detoxification of selenium in mung bean (*Phaseolus aureus* Roxb.) cuttings. Euphrates J. Agric. Sci., 5(2):107-118.

Shibagaki, N., Rose, A., McDermott, J. P., Fujiwara, T., Hayashi, H., Yoneyama, T., Davis, J.P. (2002). Selenate-resistant mutants of *Arabidopsis thaliana* identified SULTR 1; 2 a sulfate transporter required for efficient transporter sulfate into roots. Plant J., 29: 475–486.

Sors, T.G., Ellis, D.R., & Salt, D.E. (2005). Selenium uptake, translocation, assimilation and metabolic fate in plants. Photosynth. Res., 86:373–389.

Stadtman, T.C. (1996). Selenocysteine. Annul Rev. Biochem., 65: 83–100.

Stroud, J.L., Li, H.F., Lopez-Bellido, F.J., Broadley, M.R., Foot, I.,Fairweather- Tait, S.J., Hart, D.J., Hurst, R., Knott, P., Mowat, H.,Norman, K., Scott, P., Tucker, M., White, P.J., McGrath, S. P. & Zhao, F. J.(2010). Impact of sulfur fertilization on crop response to selenium fertilization. Plant Soil, 332 (1–2): 31–40.

Sutcliffe, J. F. & Baker, D.A. (1981). Plants and Mineral Salts, 2-nd ed. Edward Arnold. p.10.

Taiz, L. & Zeiger, E.(2003).Plant Physiology,3rd ed. Sinauer. Sunderland. MA.

Terry, N., Zayed, A. M., De Souza, M. P. & Tarun, A. S. (2000). Selenium in higher plants. Annul Rev. Plant Physiol. Plant Mol. Biol., 51:401-432.

White P.J., Bowen H.C. & Parmaguru P. (2004). Interactions between selenium and sulphur nutrition in *Arabidopsis thaliana.* J. Exp. Bot., 55:1927-1937.

Xiao-Zhang Y. & Ji-Dong G. (2008). Differences in uptake and translocation of selenate and selenite by the weeping willow and hybrid willow. Environ. Sci. Pollut. Res., 15:499–508.

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