SELENIUM TOXICITY IN BERSEEM (*TRIFOLIUM ALEXANDRINUM*) AND ITS DETOXICATION BY SULPHUR

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SUMMARY

The toxicity of selenium (Se) in Berseem (*Trifolium alexandrinum*) a multicut fodder crop and its detoxication by (S) was studied in pots at Haryana Agricultural University, Hissar. Se was supplied at the rate of 0, 1.0, 2.5 and 5 ppm as $Na_2SeO_3 . 5H_2O$ and S at the rate of 0, 50 and 100 ppm as K_2SO_4. The Se levels of 2.5 and 5 ppm decreased the dry fodder yield in absence of applied S, however, 1 ppm Se showed stimulation in yield. S application at 50 and 100 ppm increased dry matter yield. In presence of high S only 5 ppm Se decreaeed dry matter yield significantly. The highest dry matter yield was recorded in second cutting.

The increasing level of Se from 0 to 5 ppm increased Se concentration and decreased S, N, P and S-aminoacids, whereas S application increased S, N, P and S-aminoacids. The P concentration decreased with increase in Se when S was not applied but in presence of high S applied Se increased P concentration in plants. The decrease in S-aminoacids of plants due to Se application was not only due to decrease in S concentration but also due to decrease in N concentration as S and N showed equally high correlation values with S-aminoacids.

INTRODUCTION

Selenium is a non-essential element for plants, but it is accumulated in lethal amounts by plants from the soils which contain high available Se (Singh, 1975). It becomes toxic to animals even at a low concentration of 5 ppm in animal feeds (Anderson *et al.* 1961). Animals fed on such feeds and fodders show syndromic diseases of Se toxicity.

Although Se deficiency may also occur in animals if fed on feeds and fodders of very low Se content ($\angle$ .3 ppm) but in a country like India only a problem of toxicity could be postulated as the soils in parts of Haryana (India) show high available and total Se than average Indian soils (Singh and Kumar, 1976). Some of soils contain such high amounts of Se that if on such soil a fodder crop like berseem (*Trifolium alexandrinum*) is grown it may accumulate high Se content which would be toxic to animals. To know the actual effects of high Se on plant growth

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and its chemical composition becomes important with animal health point of view. Moreover to find out an antidote to Se so that the problem of toxicity could be avoided becomes still more important. Although, S is found to detoxicate Se due to their antagonistic relationship (Singh and Singh, 1978) in oilseed and grassy fodders, but nothing could be predicted for multicut fodders like berseem. Moreover knowing the mechanism of the action of an antidote is also needed. Keeping in all these points into consideration a pot culture experiment on the toxicity of Se and detoxication by S in berseem was conducted and the results are reported in this paper.

MATERIALS AND METHODS

The experiment was conducted on sandy soil having pH 8.0, O.C. 0.08%, EC 0.98 mmhos/cm, CEC 4.5 me/100g, available P 5.2 ppm, available N 20 ppm, available sulphur 4.2 ppm, and available selenium 0.05 ppm. The soil was passed through 2 mm sieve and filled in earthen pots of 25 cm diameter lined with polyethylene, at the rate of 4 kg per pot. Sulphur was added at the rate of 0, 50, and 100 ppm as K_2SO_4 and selenium at the rate of 0, 1, 2.5 and 5 ppm as $Na_2SeO_3.5H_2O$. Basal dose of N, P, Zn, Mn and Fe were applied at the rate of 30, 60, 5, 5, and 5 ppm, respectively. Potassium was balanced by potassum chloride. Berseem seeds (var. Muscavi) at the rate of 20 per pot were sown which after germination were thinned to 15 seedlings. Treatments were replicated four times and crop was raised under controlled condition in the screen-house using deionised water for irrigation. Three cuttings of the crop were taken after 45, 75 and 105 days of sowing.

Chopped plant material was first dried in air and then in electric oven at 60°C. Dry matter yield of individual cuttings was recorded. The dried samples were ground in stainless steel grinder. Samples were digested in nitric-perchloric acid mixture and analysed for Se, P and S by Cumins *et al.* (1965), Koenig and Johnson (1942) and Chesnin and Yien (1950) methods, respectively. Nitrogen was estimated by Nesseler's reagent (Lindner, 1944) method in sulphuric-perchloric acid digest. Sulphur containing aminoacids were extracted from 250 mg plant material by hydrolysing it in 10 ml of 5.5 N HCl at 110°C for 24 hours in sealed test tubes. The extract thus obtained was used to estimate methionine content by Horn-Jones and Blum (1946) and cystine and cysteine by Leach (1966) methods.

RESULTS AND DISCUSSION

Dry matter yield : The soil application of Se from 0 to 5 ppm in the form of $Na_2SeO_3.5H_2O$ to simulate high available Se conditions in the soil, decreased the dry matter yield of berseem fodder in Ist, 2nd and 3rd cutting significantly in absence and presence of added S as also observed by Singh and Kumar (1976). The Se addition at the rate of 1 ppm, however, tended to increase the dry fodder yield in first cut in absence and presence of high amount of S. Singh *et al.* (1980) also observed stimulation in yield of Raya with 1 ppm Se. With the increase in S to 50 and 100 ppm, the dry fodder yield increased significantly over no S treatments, but 100 ppm S did not give higher fodder yield than 50 ppm S. Unlike other crops (Singh, 1975 ; Kumar, 1978) 100 ppm S also did not decrease dry fodder yield in comparison to 50 ppm S, which was probably due to high requirement of S for berseem. The overall higher fodder yield was achieved in 2nd cutting as also reported previously (Singh and Kumar, 1976). As regards the effect of S application to over come Se toxicity, it was indicated by the data (Table 1) that when 50 ppm S was added even 2.5 Se did not decrease dry fodder yield over 1 ppm Se which was at variance with the effect of 2.5 ppm Se in absence of S. With further increase in S to 100 ppm, 2.5 ppm Se rather increased dry matter yield over 1 ppm Se. It indicated that in presence of high S high Se is tolerated and rather plays stimulating role. Singh *et al.* (1976) observed stimulation in the yield of fodder sorghum with 1, 2 and 4 ppm of Se in presence of 90 ppm and higher applied S.

Table I. Effect of sulphur and selenium on dry matter yield and selenium and phosphorus concentration in berseem

Treatments	Dry matter yield (g/pot)				Selenium (ppm)			Phosphorus (ppm)		
	Cuttings				Cuttings			Cuttings		
	I	II	III	Total	I	II	III	I	II	III
S_0Se_0	1.112	2.437	1.780	5.329	2.3	1.6	0.6	1735	1520	1305
S_0Se_1	1.202	2.413	1.786	5.401	8.4	7.2	4.3	1735	1522	1305
$S_0Se_{2.5}$	1.035	1.748	1.598	4.381	13.7	11.5	7.5	1700	1432	1217
S_0Se_5	0.628	1.065	1.276	2.969	28.2	21.5	15.2	1612	1305	1008
$S_{50}Se_0$	2.565	5.825	4.655	13.045	1.7	1.2	0.4	2535	2310	1785
$S_{50}Se_1$	2.565	5.833	4.642	13.040	7.2	5.8	2.1	2675	2435	1860
$S_{50}Se_{2.5}$	2.007	5.632	4.554	12.193	11.3	9.3	5.2	2772	2638	1905
$S_{50}Se_5$	1.072	3.985	3.051	8.108	23.8	18.5	10.7	2772	2705	1978
$S_{100}Se_0$	2.573	5.972	4.392	12.897	0.8	0.7	0.2	2610	2372	1603
$S_{100}Se_1$	2.612	5.992	4.405	13.009	4.3	3.9	0.8	2610	2410	1668
$S_{100}Se_{2.5}$	2.603	5.735	4.463	12.801	6.6	4.7	3.2	2832	2535	1705
$S_{100}Se_5$	1.852	4.385	4.082	10.319	15.3	14.2	10.4	2710	2410	1705
L.S.D. (5%)										
S	0.27	0.19	0.20		0.20	0.21	0.25	23.3	26.2	N.S.
Se	0.21	0.09	0.11		0.13	0.17	0.20	N.S.	17.6	4.6
S x Se	0.36	0.23	0.31		0.42	0.36	0.46	N.S.	N.S.	N.S.

Se concentration : The increasing levels of S from 0 to 50 and 100 ppm decreased the Se concentration from 2.3 to 1.7 and 0.8 ppm, respectively, which was due to antagonistic relationship of S and Se as also reported by Hurd Karrer (1938), and Singh and Singh (1979), but the increasing doses of Se increased Se concentration in all cuttings highest being 28.2 ppm with 5 ppm Se in first cutting. The Se concentration decreased significantly in 2nd and 3rd cutting which was mainly due to removal of a part of the available Se from the soil by first cut and transformation of some of the Se to unavailable form in the soil during crop growth period. This was also reported by Singh and Kumar (1976) in case of berseem.

P concentration : The concentration of phosphorus also increased significantly in all the three cuts with the increase in S application to 50 and 100 ppm as also observed by Broyer *et al.* (1972). Se, however, suppressed P absorption. However, increasing dose of Se decreased the P content in plants when S was not applied to the soil but when S was applied to soil the increasing dose of Se rather increased P content which was due to significant interaction between S×Se on P concentration. The differences between the effects of 50 and 100 ppm S were not as spectacular as in case of 0 and 50 ppm S. The concentration of P decreased in 2nd and 3rd cutting in comparison to 1st cutting significantly in all the treatments.

S concentration : The S concentration in 1st, 2nd and 3rd cutting decreased with increasing levels of Se in presence and absence of added sulphur. S concentration decreased in 2nd and 3rd cutting significantly in comparison to 1st cutting. The S concentration was much higher when S was applied at 50 and 100 ppm to the soil, and higher dose of Se could not decrease S concentration as much in case of 50 and 100 ppm S as in case of no S treatment. With 100 ppm S even 2.5 ppm Se did not register significant decrease in S concentration. This all indicates that even though S, and Se have strong antagonism, high levels of Se found in soils (Singh and Kumar, 1976) will not cause much harm if sufficient S is applied in such areas.

N concentration : Nitrogen concentration in first cutting ranged between 0.20 to 1.75 per cent, in 2nd cutting between 0.81 and 1.94 per cent and in 3rd cutting between 0.4 and 1.56 per cent when S was not applied. The application of 2.5 and 5 ppm Se drastically decreased the concentration of nitrogen. When S was applied at the rate of 50 ppm nitrogen concentration increased from 0.86 to 1.75 per cent but with the addition of Se from 0 to 5 ppm, nitrogen concentration decreased significantly only with 2.5 and 5 ppm. Although, the decrease was significant but much less than in case of no S. Singh *et al.* (1980) observed that Se addition decreased N content and S application increased it in raya plants. With further increase in S to 100 ppm the concentration of nitrogen increased over control from 0.86 to 1.66 but decreased against 50 ppm S from 1.75 to 1.66 per cent. At this level of S the addition of 1 and 2.5 ppm Se did not decrease or increase nitrogen concentration but with 5 ppm Se, nitrogen concentration decreased significantly. In

Table II. Effect of sulphur and selenium on the concentration of sulphur and nitrogen in berseem

Treatments	Sulphur (ppm)			Nitrogen (%)		
	I	II	III	I	II	III
S_0Se_0	935	732	639	0.86	1.12	0.68
S_0Se_1	900	719	636	0.82	1.10	0.68
$S_0Se_{2.5}$	805	613	547	0.62	1.00	0.62
S_0Se_5	572	504	412	0.20	0.81	0.40
$S_{50}Se_0$	1750	1607	1322	1.75	1.84	1.56
$S_{50}Se_1$	1750	1605	1322	1.75	1.94	1.55
$S_{50}Se_{2.5}$	1630	1416	1209	1.55	1.73	1.34
$S_{50}Se_5$	1447	1116	810	1.23	1.55	1.20
$S_{100}Se_0$	2238	2232	2016	1.66	1.63	1.23
$S_{100}Se_1$	2245	2232	2020	1.67	1.64	1.24
$S_{100}Se_{2.5}$	2230	2205	2013	1.70	1.80	1.33
$S_{100}Se_5$	2016	2007	1939	1.31	1.62	1.20
L.S.D. (5%)						
S	26.3	22.5	N.S.	0.20	0.32	0.11
Se	20.5	13.3	N.S.	0.16	0.23	N.S.
S x Se	45.7	47.0	N.S.	0.40	N.S.	N.S.

case of 2nd cutting the concentration increased more than 30 per cent but in III cutting again decreased to less than 1st cutting. Although in case of no S treatment the trend of decrease in nitrogen with Se was similar as in case of 1st cutting but with 50 ppm S, 1 ppm Se rather increased nitrogen concentration in 2nd cutting. Rest of the treatment in 2nd cutting and all concentrations of Se in 3rd cutting with 50 ppm S were similar as in 1st cutting. With 100 ppm S, however, Se application upto 2.5 ppm tended to increase nitrogen concentration in all cuttings. (Table II).

N : S ratio : The N : S ratio tended to increase with increase in S to 50 ppm showing that increase in nitrogen proportion was even more than S but with further increase in S to 100 ppm N : S ratio decreased indicating that S concentration increased and N decreased in comparison to 50 ppm S. Se application in all cases decreased N : S ratio indicating that N was more severely affected than S by Se addition. Unlike Aulakh and Pasricha (1977) N : S ratio in this case ranged between 3.4 to 10.

S-Aminoacid concentration : The S-aminoacids, viz., methionine, cystine and cysteine increased with the application of S in all the cuttings. The concentration in all the cuttings ranged between 12 to 28 mg/g N (methionine), 20 to 32 mg/g N (cystine) and 12 to 19 mg/g N (cysteine). The general trend in the effects of S and Se application was almost same in all cases where 50 ppm S increased aminoacid content against control, (Table III). However, 100 ppm S tended to decrease against cystine and cysteine content in first cutting in comparison to 50 ppm S. Se application at all rates decreased aminoacids in no S treatments but when S was added at 50 ppm and 100 ppm, significant decrease came only with 5 ppm Se. Methionine in 1st cutting showed more drastic decrease than other cases. Normally 2nd cutting showed higher concentration and less drastic effects. Singh and Singh (1979) and Singh and Singh (1978) observed decrease in S-aminoacids in raya and wheat plants by the application of Se and increase in S aminoacid by the application of S. It was also observed that the S-aminoacid content was less with 100 ppm S than with 50 ppm S as in the present case.

The positive and highly significant correlation values between S and aminoacid contant, N and amino acid content and negative significant values between N : S ratio and S-aminoacid content (Table IV) indicate that the synthesis of aminoacid is highly dependent on the availability of S and N in the plants and both the factors are equally strongly related to aminoacid content. The decrease in S-aminoacid with Se application was not only due to the decrease in S content due to their antagonistic relationship but also due to drastic decrease in N content. Similarly, the increase in S-aminoacid was probably not only by increased supply of S but also because of increase in N content by S application as indicated by high and positive correlation between N and S.

Table III. Effect of S and Se on S-aminoacids in berseem

Treatment	Methionine (mg/g N)			Cystine (mg/g N)			Cysteine (mg/g N)		
	Cuttings			Cuttings			Cuttings		
	I	II	III	I	II	III	I	II	III
S_0Se_0	17.8	23.4	17.6	23.6	24.6	21.2	15.5	16.2	14.3
S_0Se_1	17.0	22.6	17.6	23.0	24.5	21.2	15.6	16.0	14.3
$S_0Se_{2.5}$	15.1	18.3	16.2	22.1	24.6	21.0	14.3	15.8	14.1
S_0Se_5	12.4	15.8	16.0	20.4	24.6	20.6	12.6	15.3	14.0
$S_{50}Se_0$	25.4	27.1	20.3	30.5	31.2	26.3	28.4	18.6	16.8
$S_{50}Se_1$	25.5	27.1	20.6	30.4	31.6	26.2	18.1	18.7	16.9
$S_{50}Se_{2.5}$	23.4	27.0	20.0	26.2	31.0	26.0	17.8	18.2	16.2
$S_{50}Se_5$	20.4	23.2	20.0	24.0	29.3	25.6	15.8	18.0	16.0
$S_{100}Se_0$	26.4	27.8	21.2	30.1	30.8	27.3	18.2	18.8	17.3
$S_{100}Se_1$	26.6	27.8	21.3	30.4	30.8	27.6	18.3	18.8	17.5
$S_{100}Se_{2.5}$	26.4	27.2	21.0	30.4	30.0	27.0	18.3	18.6	17.0
$S_{100}Se_5$	24.2	26.5	27.0	27.3	30.0	26.7	17.4	18.2	16.8
L.S.D. (5%)									
S	0.23	0.12	0.11	0.17	0.21	0.18	0.13	N.S.	0.26
Se	0.14	0.05	N.S.	0.11	0.16	N.S.	0.07	N.S.	N.S.
S x Se	0.32	0.21	N.S.	N.S.	0.31	0.31	0.22	N.S.	N.S.

Table IV. Correlation coefficients between S, N, N : S and S-aminoacids in berseem

Factors correlated	Cuttings		
	I	II	III
Sulphur x Nitrogen	0.908**	0.818**	0.713**
Sulphur x Methionine	0.967**	0.860**	0.904**
Sulphur x Cystine	0.921**	0.853**	0.903**
Sulphur x Cysteine	0.913**	0.913**	0.925**
Nitrogen x Methionine	0.979**	0.904**	0.901**
Nitrogen x Cystine	0.949**	0.687*	0.676*
Nitrogen x Cysteine	0.981**	0.691*	0.671*
N : S ratio x Methionine	0.398	—0.814**	—0.393
N : S ratio x Cystine	0.374	—0.792**	—0.3[illegible]
N : S ratio x Cysteine	0.536	—0.858**	—0.436

* Significant at 5% level of significance

** Significant at 1% level of significance

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