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SELENIUM TOXICITY IN PLANTS AND ITS DETOXICATION BY PHOSPHORUS

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ABSTRACT

Selenium toxicity and the effect of P application on Se in wheat (*Triticum aestivum*) was studied in a greenhouse experiment using the sandy soil of Haryana Agricultural University, Hissar, India. Selenium was applied at 0, 2.5, 5, and 10 ppm as $Na_2SeO_3 \cdot 5H_2O$, and P at 0, 50, and 100 ppm as KH_2PO_4. The application of P at 50 ppm increased, and that at 100 ppm decreased, dry matter, grain yield, N, S, Zn, Cu, chlorophyll, DNA, RNA, protein, and proline contents, and S-amino acids in wheat. Contents of Fe and Mn decreased as P increased to 50 and 100 ppm. Plant P increased with increases in added P up to 100 ppm. In P control and 50 ppm P treatments, the addition of Se decreased dry matter yield, grain yield, N, Zn, Cu at 50 days and maturity (135 days), DNA, RNA, protein, and proline in plants at 45 and 90 days after planting, and S-amino acids in grains, but 2.5 ppm Se with 50 ppm P increased, P, S, Fe, and Mn, and 2.5 and 5 ppm Se with 100 ppm P were beneficial on almost all parameters. Se content increased with 50 ppm P and all concentrations of Se, but decreased with 100 ppm P. Free amino acids were greater at 90 days than at 45 days sampling. Phosphorus application decreased free amino acids, but Se increased them.

Sunflower (*Helianthus annuus*) was grown after wheat to see the residual effect of Se and P. In sunflower there was low accumulation of Se, which increased with high Se and 50 ppm P, and decreased with 100 ppm P applied to the wheat crop. But, in this case, 50 and 100 ppm P added to the wheat crop increased N, P, and S. At 100 ppm P added to wheat, 2.5 and 5 ppm Se showed increased dry matter, P, and S content at 90 and 135 days.

The high amounts (2.5 ppm and above) of added and residual Se in soil decreased the growth of wheat and sunflower, the absorption of Zn, Cu, Fe, Mn, S, and N, and the synthesis of nucleic acids, chlorophyll, and protein, whereas P decreased the harmful effect of Se, and increased all the above parameters.

INTRODUCTION

Selenium has been recognized as an essential element for all the animals (Allaway and Cary 1964), but only for one group of plants, i.e., Astragalus species (Trelease and Trelease 1938). It may be absorbed by plants in lethal amounts, depending on various soil and plant factors that depress plant growth and alter metabolic activities about which little is known. Selenium stimulates plant growth (Singh, Bhandari, Singh 1976), which may be due to its substitution in definite enzymic paths of some essential nutrients like sulfur. Sulfur is, therefore, found to be an antidote to the toxicity of selenium (Singh 1977). Although phosphorus was also found to detoxicate selenium toxicity in some plants (Singh and Bhandari 1974), the definite mechanism of its action is not known. A detailed review of selenium in soils and plants has been presented by Ganje (1973).

Soils in some parts of Haryana, India have high contents of available selenium, and large amounts of selenium are accumulated by such crop plants as sorghum and paddy, which may

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create toxicity in plants and animals. Since P was found to reduce the harmful effects of Se (Singh and Kumer 1976), although the mechanism of action was not known, the present study was conducted in pots, under controlled conditions in the greenhouse, and detailed analysis of plants was reported.

EXPERIMENTAL

The experiment was conducted in earthen pots (25 cm in diameter and 25 cm high) lined with polyethylene in the Department of Soils, Haryana Agricultural University, Hissar, India. The soil was Rawalwas sand, which had pH (1:2), 7.9 EC 1.0 mmhos/cm, O.C. percent 0.1, CEC 3.25 me/100g, as determined by the methods outlined by Jackson (1958). Available Se (hot water-soluble) was 0.05 ppm, available (Olsen's) P and heat soluble S were 4 ppm each. The DTPA-extractable Fe, Mn, Zn, and Cu were 1.17, 0.92, 0.12, and 0.19 ppm, respectively. Air-dry soil, which was crushed and passed through a 2-mm sieve, was placed in the pots. Selenium was applied at the rate of 0, 2.5, 5, and 10 ppm as $Na_2SeO_3 \cdot 5H_2O$, and P was added at the rate of 0, 50, and 100 ppm as KH_2PO_4 (five replications) through analytical grade reagents. Nitrogen was applied at the rate of 100 ppm through reagent grade urea. Zinc, Fe, and Mn were applied at 5 ppm, Cu was applied at 2.5 ppm through the sulfates, and S was made up by K_2SO_4 at the rate of 25 ppm. A wheat variety, Kalyan sona (*Triticum aestivum*), was sown at the rate of four healthy seeds per pot, and was thinned to two uniform plants after germination. Water, 100 ml every other day, was added to each pot to meet the full requirement of the crop. The green leaf analysis for chlorophyll, DNA, and RNA was done at 45 and 90 days; to determine other parameters, crops were harvested at 50 days and maturity (135 days after planting).

After the wheat was harvested, sunflower (*Helianthus annuus*) was grown, one plant per pot for 30 days in three replications to learn the residual effect. The plants were harvested after 30 days and prepared for analysis.

Oven-dried and crushed samples were digested in HNO_3:$HClO_4$, and analyzed for P by the Koenig and Johnson (1942) method, for Se by the method of Cummins et al. (1964), and for Cu, Fe, Zn, and Mn by a Varian Tetron Model 120 A atomic absorption spectrophotometer. Selenium was estimated in wet oxidized sample (Johnson and Nishita 1952) by the turbidimetric method (Chesnin and Yien 1950). Methionine, cysteine, and cystine were extracted from grain in 6 *N* HCl. Methionine was analyzed by the method of Horn, Jones, and Blum (1946), and cystein and cystine by the Leach method (1968). Plants were also digested in H_2SO_4:$HClO_4$, and N was estimated by Nessler's reagent method. Chlorophyll was extracted from 200 mg fresh leaves, in 20 percent ethanol, until colorless residue was left. The optical density was recorded at 665 mu on an ultraviolet spectrophotometer model, taking 80 percent ethanol as the blank. Nucleic acids were estimated by the method of Nieman and Poulsen (1963). Free amino acids were estimated by the Barnet and Naylor method (1966), and protein by the Folin phenol reagent method of Lowry et al. (1951). Free proline was estimated by the method of Bates, Waldren, and Teare (1973). The data were analyzed statistically and significance was measured by Fisher's test.

RESULTS AND DISCUSSION

Dry matter and grain yield of wheat

The dry matter at 50 days and maturity decreased significantly with the increase in Se in the growth medium from 0–10 ppm. The decrease in dry matter yield over the control, with 10 ppm Se, was more than three to five times, whereas grain yield decreased up to ninefold (Table 1). The application of P at 50 ppm increased dry matter yield twofold at 50 days, and maturity and grain yield about threefold. The increasing Se level, even with 50 ppm P, showed a similar trend. With a further increase in P, to 100 ppm, however, P tended to depress the dry matter and grain yield over 50 ppm, but an increasing level of Se stimulated the dry matter yield up to 5 ppm and grain yield up to 2.5 ppm. Singh and Bhandari (1974) and Singh and Kumar (1976) also observed similar results in pea and berseem.

Nitrogen and phosphorus

The application of Se decreased N content twofold at 50 days and about threefold at maturity, as also reported by Singh (1977). The addition of 50 and 100 ppm P increased N content at both times significantly. There was, however, a depression in N content with 100 ppm P over 50 ppm P. The trend of Se response was

TABLE 1

Effect of Se and P on dry matter, grain yield, and N, P, S, and Se contents

Treatments (1)	50 days sampling after sowing					Maturity					
	D.M.Y., g/pot (2)	Nitrogen, % (3)	Phosphorus, ppm (4)	Sulfur, ppm (5)	Selenium, ppm (6)	D.M.Y., g/pot (7)	Grain yield, g/pot (8)	Nitrogen, % (9)	Phosphorus, ppm (10)	Sulfur, ppm (11)	Selenium, ppm (12)
P_0Se_0	2.3	0.45	1632.5	1637	0.4	6.4	1.8	0.74	1173.2	1026	0.6
$P_0Se_{2.5}$	1.8	0.40	1425.2	1406	13.5	5.0	1.3	0.62	938.5	835	10.2
P_0Se_5	1.5	0.35	1106.6	1036	28.2	4.2	0.3	0.59	665.2	378	24.5
P_0Se_{10}	0.5	0.22	542.6	585	43.7	1.8	0.2	0.27	217.7	209	36.8
$P_{50}Se_0$	4.5	0.54	2454.0	2358	0.7	14.1	5.3	1.05	2136.6	1508	0.6
$P_{50}Se_{2.5}$	3.6	0.50	2708.4	2432	15.6	11.8	4.8	1.00	2443.4	1715	11.2
$P_{50}Se_5$	2.3	0.42	2691.7	2066	32.3	9.4	3.4	0.94	2262.5	1376	27.2
$P_{50}Se_{10}$	1.2	0.28	2026.0	1646	53.5	2.4	0.5	0.78	1738.5	1052	42.6
$P_{100}Se_0$	3.8	0.50	3375.2	2048	0.3	13.4	4.4	0.94	2436.8	1357	0.08
$P_{100}Se_{2.5}$	4.0	0.46	3512.2	2205	7.5	15.4	4.7	0.90	2564.5	1423	5.3
$P_{100}Se_5$	4.2	0.48	3836.5	2232	26.8	15.6	3.4	0.84	2832.3	1406	22.6
$P_{100}Se_{10}$	2.4	0.25	3120.3	1974	46.3	5.6	1.3	0.66	2384.6	1258	39.3
C.D. at 5% (P)	0.16	0.16	32.8	38.6	0.21	0.28	0.22	0.20	21.8	26.6	0.21
(Se)	0.07	0.11	22.3	27.2	0.17	0.20	0.14	0.11	20.3	18.9	0.15
(P × Se)	0.20	0.41	NS	64.6	0.41	0.35	0.29	0.41	NS	52.2	0.31

the same in both the presence and the absence of P. There was a drastic reduction in P content with Se application at both the intervals. The P content at maturity was much lower than at 50 days, which was obviously due to dilution. The application of P at 50 and 100 ppm increased P content in plants from one and a half to over two times. The behavior of Se in the presence of high P contents changed during the growth period. The P content in the plants increased at both times with increasing Se up to 5 ppm in the growth medium. With only 10 ppm Se, P content was reduced dramatically when 100 ppm P was supplied to the plants. All the individual and interaction effects were significant.

Sulfur and selenium

The S and Se concentrations in wheat plants (Table 1) were much higher at 50 days than at maturity, except for Se in P_0Se_0 treatment. With the application of Se alone, from 0 to 10 ppm, S in plants decreased about threefold at 50 days and about fivefold at maturity, whereas Se increased more than 100 times at 50 days and 60 times at maturity. The application of 50 ppm P increased S, as well as Se significantly over plants that had no P, whereas 100 ppm P decreased both S and Se. In the presence of 100 ppm P, the application of 2.5 and 5 ppm Se increased plant Se at 50 days, but, at maturity, only 2.5 ppm Se increased it over plants without added Se. In the case of 50 ppm P application, only 2.5 ppm Se increased plant sulfur over the Se treatment. The higher doses of Se (5 and 10 ppm), when applied with 100 ppm P, showed higher plant S in comparison to 50 ppm P, which indicated significant interaction between Se and P on plant S. Singh (1977), Singh, Bhandari, and Singh (1976), and Singh and Kumar (1976) observed similar P and Se interaction and Se and S antagonism in other crops. The increasing application of Se increased plant Se significantly at all levels of P treatments.

Micronutrients

Zinc, Cu, Mn, and Fe were higher in plants at 50 days than at 135 days in all the treatments (Table 2). Selenium application significantly decreased the plant content of Zn, Cu, Mn, and Fe at 50 days and 135 days of crop growth. Broyer, Johnson, and Huston (1972) observed significant decrease in micronutrient cations by the increased amounts of Se in growth medium in alfalfa. The decrease with 10 ppm applied Se was so drastic that the Zn levels in plants decreased to very low levels (5.4 ppm). The application of P, at the rate of 50 ppm, increased the Zn and Cu contents in plants, but decreased Fe and Mn content over no P treatment. Nevertheless, P, at 100 ppm, decreased all four micronutrients significantly over 50 ppm P and no P. In P_0 and P_{50} treatments, increasing levels of Se decreased Zn and Cu, but, in P_{100} treatment there were larger amounts of Zn, Cu, Mn, and

TABLE 2

Effect of Se *and* P *on* Zn, Cu, Fe, *and* Mn *contents in plants*

Treatments	50 days sampling after sowing				135 days (maturity)			
	Zn, ppm (1)	Cu, ppm (2)	Fe, ppm (3)	Mn, ppm (4)	Zn, ppm (5)	Cu, ppm (6)	Fe, ppm (7)	Mn, ppm (8)
P_0Se_0	15.2	8.7	275.3	58.8	11.6	5.8	205.0	49.6
$P_0Se_{2.5}$	13.5	7.2	256.5	56.0	10.2	5.2	170.3	38.8
P_0Se_5	9.2	5.8	236.0	50.0	7.2	4.3	156.6	30.2
P_0Se_{10}	5.8	5.1	210.6	46.0	3.5	4.0	130.2	25.2
$P_{50}Se_0$	18.5	13.2	240.2	58.0	15.3	8.4	180.3	46.8
$P_{50}Se_{2.5}$	17.2	12.8	257.2	58.5	15.0	8.3	186.5	46.0
$P_{50}Se_5$	15.3	11.2	250.5	57.2	11.5	7.5	165.2	44.2
$P_{50}Se_{10}$	12.2	8.2	235.7	54.0	8.2	5.2	135.3	40.5
$P_{100}Se_0$	13.0	10.3	203.3	52.4	10.0	7.2	160.5	42.3
$P_{100}Se_{2.5}$	14.2	10.4	216.2	54.6	10.7	7.8	167.6	44.8
$P_{100}Se_5$	16.4	10.4	230.8	55.5	11.5	8.4	173.2	45.2
$P_{100}Se_{10}$	11.8	8.6	230.5	50.0	13.2	7.7	178.7	43.4
C.D. at 5% (P)	2.7	2.1	31.1	NS	1.9	1.1	30.6	NS
(Se)	2.1	1.2	20.7	NS	1.0	0.7	19.2	NS
(P × Se)	4.6	NS	52.6	NS	3.8	NS	60.5	NS

Fe in Se at 2.5 and 5 ppm than at Se_0 (Table 2, cols. 1–4). The trends at 50 days and maturity were similar. The differences in Mn content due to Se and P application (Table 2, cols. 4 and 8) were not significant.

Sulfur-containing amino acids in grain

Cystine and cysteine in wheat grains (Fig. 1, B and C) increased significantly with 50 and 100 ppm P, but methionine (Fig. 1, A) increased with 50 ppm and decreased with 100 ppm P over 50 ppm P. Selenium application decreased all S-amino acids significantly. This is attributed to the replacement of S by Se in the constitution of amino acids. Singh (1977) observed decrease in S-amino acids in *Brassica juncea* Cos. plants by the addition of Se to soil. In the case of 100 ppm P, the presence of 5 ppm Se was not at all harmful, and even 10 ppm Se was only slightly harmful for methionine and cystine. In the case of cystine, however, 100 ppm P plus 5 ppm Se was also harmful. Methionine, cystine, and cysteine ranged from 11.2 to 16.3, 8.6 to 18.6, and 3.2 to 13.7 ppm, respectively.

Chlorophyll content

The chlorophyll content (Table 3) in all the treatments was higher at 45 days than at 90

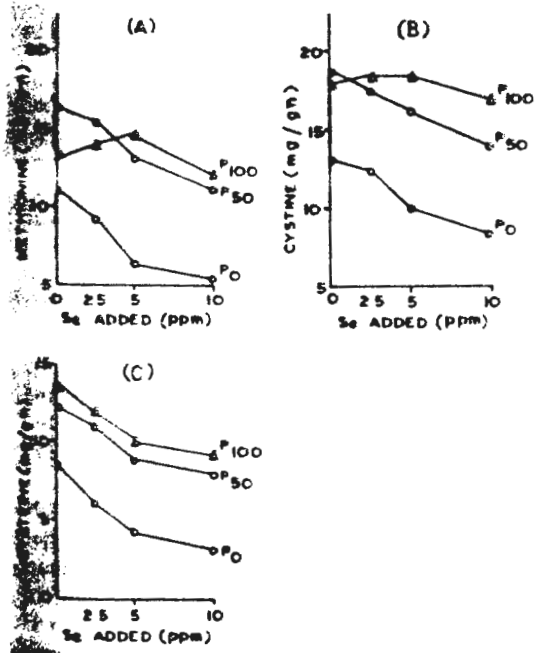

FIG. 1. (A) Methionine; (B) cystine; (C) cysteine in wheat grains.

TABLE 3

Effect of Se and P on chlorophyll, DNA, RNA, protein, proline, and free amino acids contents

Treatments	45 days sampling after sowing							90 days sampling after sowing					
	Chlorophyll, (T) (1)	RNA, mg/g (2)	DNA, mg/g (3)	Protein, mg/g (4)	Proline, µg/g (5)	Free amino acids, µg/g (6)	Chlorophyll, (T) (7)	RNA, mg/g (8)	DNA, mg/g (9)	Protein, mg/g (10)	Proline, µg/g (11)	Free amino acids, µg/g (12)
P_0Se_0	0.810	1.306	0.446	11.56	81.11	64.2	0.623	1.160	0.442	10.32	71.36	73.2
$P_0Se_{2.5}$	0.796	1.052	0.446	9.49	73.73	86.5	0.607	1.010	0.432	8.08	64.25	92.5
P_0Se_5	0.721	0.703	0.302	7.30	45.36	115.5	0.550	0.560	0.265	5.68	42.67	135.2
$P_{50}Se_0$	0.886	2.460	1.653	12.58	127.12	55.2	0.670	1.983	1.658	12.36	120.23	63.2
$P_{50}Se_{2.5}$	0.870	2.433	1.655	11.73	125.45	78.6	0.650	1.934	1.650	12.00	120.29	86.2
$P_{50}Se_5$	0.854	2.015	1.625	9.69	110.30	90.3	0.623	1.554	1.526	8.16	107.64	105.6
$P_{100}Se_0$	0.870	2.256	1.505	10.83	98.85	47.5	0.721	1.915	1.645	9.86	88.34	51.5
$P_{100}Se_{2.5}$	0.886	2.478	1.518	12.25	107.57	52.5	0.886	1.982	1.653	10.48	93.25	68.3
$P_{100}Se_5$	0.854	2.113	1.515	11.05	99.86	60.7	0.714	1.632	1.625	10.12	92.07	76.5
C.D. at 5% (P)	0.025	0.026	NS	0.324	10.04	—	0.018	0.020	NS	0.215	8.23	—
(Se)	0.020	0.017	NS	0.230	7.21	—	0.015	0.016	NS	0.210	6.42	—
(P × Se)	0.046	0.030	NS	0.632	18.14	—	0.037	0.040	NS	0.432	16.26	—

days. The application of 50 and 100 ppm P to soil increased chlorophyll content in plants at both stages of sampling, probably due to increase in plant N and S. The increase in Se in the growth medium, from 0 to 5 ppm, decreased the chlorophyll content at 0 and 50 ppm P, but at 100 ppm P, 2.5 ppm Se stimulated chlorophyll content.

Nucleic acids

DNA and RNA are involved in the synthesis of proteins. DNA in plants did not vary at the two stages. The application of 50 ppm P increased DNA content significantly, but 100 ppm P had a tendency to decrease the DNA over 50 ppm P. Applying 2.5 ppm Se did not affect DNA, but 5 ppm Se decreased DNA at both stages when no P and 50 and 100 ppm P was added. At 45 days, however, the lower dose, 2.5 ppm Se, with 100 ppm P increased the DNA content over Se_0 with 100 ppm P. There were more differences in RNA than in DNA at the two stages, and both were higher at 45 days than at 90 days. Phosphorus at 50 ppm increased RNA over no P, but decreased it at 100 ppm, in comparison with 50 ppm P. The increase in DNA and RNA occurred because P is the constituent of both, although RNA was more affected than DNA. Selenium, at 2.5 and 5.0 ppm, decreased RNA when no P or 50 ppm P was applied, but, with 100 ppm P, 2.5 ppm Se showed more DNA and RNA than no Se treatment (Table 3). Lee, Caldwell, and Clapp (1966) observed that RNA content increased with increased P, but with the highest amount of P (13.0 ppm), RNA decreased. DNA was less affected than RNA.

Protein content

The protein increased with increased P levels, to 50 ppm, at both stages, but, with 100 ppm P, there was reduction in protein content over 50 ppm P. Selenium application decreased protein content with no P and 50 ppm P, but, with 100 ppm P, 2.5 ppm Se increased protein content. The increase in protein by P was due to increased N (Table 1) and nucleic acids (Table 3), whereas with Se, the reduction was due to decreased N content and also because Se checks the incorporation of amino acids into proteins (Peterson and Butler 1962).

Free amino acids

Free amino acids (Table 3) were higher at 90 days than at 45 days, indicating that protein formation at 90 days was not keeping pace with free amino acid production. The increase in P application, to 50 and 100 ppm P, decreased the free amino acid content at both stages, indicating that P helps in the formation of proteins from free amino acids. Unlike P, the increase in Se in growth medium increased the free amino acids at both times of sampling and with all the P combinations. Peterson and Butler (1962), however, reported that Se was extensively incorporated in proteins in wheat, although they further reported that plants have preference for S-amino acids against Se-amino acids. It is evident from the above discussion that Se decreases metabolism in the plant, which decreased N, S, micronutrients, chlorophyll, RNA, DNA, and protein.

Proline content

Proline, which is an amino acid and a constituent of protein, increased with 50 ppm P application over no P, but, with 100 ppm, it decreased over the 50 ppm P treatment. The application of Se at 2.5 and 5 ppm decreased proline content when no P and 50 ppm P was added, but, when 100 ppm P was added, 2.5 ppm Se increased the proline content, and 5 ppm decreased it. The effects and interactions were statistically significant.

Sunflower

In the case of the residual effect of P and Se on dry matter yield of sunflower, 50 ppm P slightly increased it, as compared to no P, but 100 ppm drastically decreased it. Selenium, at the rates of 2.5, 5, and 10 ppm added to the wheat crop, decreased dry matter yield of sunflower significantly over no Se, when no P was added, but, when 50 ppm P was added, only 5 and 10 ppm Se decreased the growth. With 100 ppm P treatment, the residual Se increased the growth and even with 10 ppm Se, dry matter yield was higher than P_0Se_0 (control). Nitrogen, P, and S increased with 50 and 100 ppm P added to the previous crop. Plant P, in 50 ppm, added P to wheat, and N, P, and S, in 100 ppm added P, increased with 2.5 and 5 ppm S, and decreased with 10 ppm Se. Selenium effects on plant N and P were nonsignificant. Plant Se was higher in those pots where Se was added to wheat than where Se was not added. Phosphorus, at 50 ppm slightly increased Se content, but 100 ppm P decreased it. The Se accumulated in sunflower was less than in wheat, probably due to a decrease in available Se, as some of the added S

TABLE 4

Effect of Se and P on dry matter yield, N, P, S, and Se in sunflower plants

Treatments, ppm	Dry matter, g/pot (1)	N, ppm (2)	P, ppm (3)	S, ppm (4)	Se, ppm (5)
P_0Se_0	1.3	0.31	1215	1056	—
$P_0Se_{2.5}$	1.1	0.28	1013	864	7.3
P_0Se_5	0.7	0.23	863	564	12.5
P_0Se_{10}	0.2	0.14	535	326	20.4
$P_{50}Se_0$	1.5	0.42	1532	1325	0.1
$P_{50}Se_{2.5}$	1.5	0.43	1685	1211	8.4
$P_{50}Se_5$	1.2	0.40	1657	1053	13.5
$P_{50}Se_{10}$	0.8	0.36	1362	705	22.5
$P_{100}Se_0$	2.2	0.51	1772	1513	—
$P_{100}Se_{2.5}$	2.3	0.54	1812	1608	6.5
$P_{100}Se_5$	2.0	0.54	1883	1626	10.2
$P_{100}Se_{10}$	1.4	0.46	1634	1352	17.6
C.D. at 5% (P)	0.11	NS	12.3	19.1	3.21
(Se)	0.05	NS	NS	16.3	NS
(P × Se)	NS	NS	NS	NS	NS

was taken by wheat and some might have been converted to unavailable forms (Table 4).

It is concluded from the above results that Se decreased yield and caused general inhibition in plants, but P, at 50 ppm, was sufficient to overcome the harmful effect of 5 ppm Se, although 100 ppm could not remove the harmful effect of 10 ppm Se fully. Selenium (2.5 ppm) in soil, added to the previous crop, was able to decrease the yield of sunflower considerably.

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