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EFFECT OF *PISUM SATIVUM* SEED PRETREATMENT WITH NICKEL ON SEED GERMINATION, SEEDLING GROWTH TOTAL N & P DISTRIBUTION[1]

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ABSTRACT

The studies on effects of seed pretreatments with nickel sulphate show the existence of dose related cultivar specific and organ specific differences in response to the heavy metal Nickel. While lower concentration promote germination, seedling growth and total N and P mobilization from cotyledons, higher concentrations, inhibit these processes. These parameters may be used for scanning of heavy metal resistant cultivars for cultivation in polluted areas.

INTRODUCTION

Reports of nickel as a heavy metal pollutant have been extensively reviewed (Mishra and Kar, 1974 ; Nriagu, 1980; and Singh, 1981). Previous studies, in this laboratory on several heavy metal pollutants as lead (Kumar, 1978 ; Banerji and Kumar, 1979), cadmium (Jain 1981), Mercury (Sharma, 1980) and Zinc (Bhargava, 1981) had indicated dose related, organ specific and cultivar specific differences to exist in the response of crop plants to heavy metal administration. It was, therefore, of interest to extend these findings with regard to Nickel.

MATERIALS AND METHODS

Seeds of three *Pisum sativum* cultivars Arkel, Bonneville and T-163 were procured from Indian Agricultural Research Institute, New Delhi. Seeds selected for uniformity (criteria being the size and colour of seeds) were surface sterilized with 0.1 % mercuric chloride solution, thoroughly washed and air dried. These seeds were soaked in solution of nicke sulphate containing 1.70×10^{-4} M and 8.52×10^{4} M, Nickel (10 mg Ni and 50 mg Ni/1), for about 24 hr. Seeds soaked in distilled water simultaneously for 24 hr constituted the control sets. Thereafter, the seeds were washed and transferred on wet blotting paper in petriplates for germination and seedling growth in dark. Germination percentage was recorded after 24 hr. For seedling growth analysis 3, 5 and 7 day old seedlings were taken and dissected into radicle, epicotyl and cotyledon (residual). Length, fresh weight and dry weight of radicle and epicotyl ; fresh weight and dry weight of residual cotyledons were measured. Observations are statistically analysed and differences in the growth observed were tested for significance using the statistic 't'.

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Total nitrogen was determined by microkjeldahl digestion with H_2SO_4 and H_2O_2 (Snell and Snell, 1954) and estimation of the digest with Nessler's reagent of Koch and Mc Meekin's formula (Oser, 1965). Total phosphate was determined by a modified Allen's method (Allen, 1940) with metol reagent (Plummer, 1971).

RESULTS AND DISCUSSION

Seed germination : Table I shows the results of the effects of nickel on germination of seeds of *Pisum sativum* cvs Arkel,

TABLE I

EFFECTS OF NICKEL ON GERMINATION OF *Pisum sativum* CULTIVARS

Cultivars	Nickel Concentration, mg/l		
	0 (Control)	10 (% germination)	50
Arkel	85.76	98.10	70.82
Bonneville	91.88	95.99	82.20
T-163	90.43	98.65	78.12

Bonneville and T-163. In all the three cultivars there is tendency of promotion of germination at 1.70×10^{-4} M, Ni concentration and inhibition at 8.52×10^{-4}M Ni concentration. However, extents of promotion and inhibition vary with the three cultivars. Germination of cv Arkel is much more inhibited and promoted as compared to Bonneville and T-163. Thus, germination percentage wise cv. Arkel seem to be more sensitive than cv. Bonneville and cv T-163. Cv Bonneville is more resistant to nickel toxicity than other cultivars.

Seedling growth : Like germination, seedling growth of *Pisum Sativum* culivars is also promoted at the lower concentration of Ni i.e. 1.70×10^{-4}M and inhibited at the higher concentration of Ni i.e. 8.52 $\times10^{-4}$M (Table II). All the parameters studied (length and fresh weight of radicle and epicotyl) show inhibition at higher concentration and promotion at lower concentration. However, organ specific varietal differences exist in the response of different cultivars to nickel pretreatment to seeds. At lower concentration of nickel in cvs. Arkel, Bonneville T-163, the growth of radicle, both length and fresh weight wise, is much more promoted as compared to the growth of epicotyl and at higher Ni concentration, the growth of epicotyl (both length and fresh weight wise) is more inhibited than the growth of radicle. By Ni treatment radicle and epicotyl, both length and fresh weight-wise, show maximum promotion in cv Arkel and minimum in cv Bonneville, cv T-163, lies inbetween. Thus, on the basis of seedling growth, cv Arkel seems to be most susceptible and cv Bonneville is most resistant. Observations also indicate that pattern of growth are not altered by Ni, although, extents of growth vary.

Results given in Table III show that the rise in dry weight of radicle and epicotyl is paralleled by decrease in cotyledon dry weight. The pattern of dry matter transfer from cotyledon to seedling parts is not altered by Ni, however, extents of dry matter increase/decrease vary with different cultivars.

Total Nitrogen and Phosphate distribution: Table IV shows the nitrogen content of radicle, epicotyl and cotyledon at 3rd 5th and 7th day of growth of three cultivars of *Pisum sativum* Nitrogen content of radicle and epicotyl is increased with the age of seedlings in all the sets and cultivars and N content of cotyledons decreased with the age of seedlings. At 1.70×10^{-4}M, Ni concentration, there is consideable increase in N content of radicle and epico-

TABLE II

EFFECTS OF NICKEL PRETREATMENT TO SEEDS ON GROWTH OF *PISUM SATIVUM* CULTIVARS SEEDEINGS IN DARK

Treatment (M Nickel)	Radicle						Epicotyl					
	Length (cm.)			Fresh weight (mg)			Length (cm.)			Fresh weight (mg)		
	Days after radicle emergence											
	3	5	7	3	5	7	3	5	7	3	5	7
CV ARKEL												
0 (Control)	3.21 ±0.65	5.73 ±1.00	8.93 ±1.12	61.80 ±5.15	91.50 ±8.35	164.20 ±10.14	1.57 ±0.35	3.20 ±0.95	6.26 ±1.50	51.20 ±3.46	110.50 ±5.27	221.60 ±15.35
1.70 × 10⁻⁴	5.14** ±1.15	8.92** ±1.30	12.14** ±2.06	91.80** ±5.00	120.80** ±7.56	209.80** ±18.44	2.42** ±0.30	3.80* ±0.45	8.96** ±1.00	75.10** ±5.64	124.70** ±6.43	282.50** ±10.46
8.52 × 10⁻⁴	1.87** ±0.42	4.40* ±0.95	6.99* ±1.00	42.70** ±4.65	80.20** ±5.10	139.80 ±8.75	0.74** ±0.20	2.50** ±0.50	3.86** ±1.05	33.20** ±3.46	83.70** ±4.35	140.60** ±12.35
CV BONNEVILLE												
0(Control)	4.36 ±0.40	8.44 ±1.00	12.61 ±0.50	71.30 ±4.00	141.10 ±5.35	255.40 ±5.46	1.14 ±0.25	4.08 ±0.50	7.23 ±0.65	31.70 ±1.50	149.10 ±4.37	322.10 ±10.65
1.70 × 10⁻⁴	5.51** ±0.50	10.03** ±0.95	13.86** ±0.60	82.50** ±5.15	167.20** ±6.36	273.50** ±8.43	1.36* ±0.35	4.28* ±0.30	8.46** ±0.50	34.49** ±2.00	160.60** ±5.43	363.90** ±15.35
8.52 × 10⁻⁴	3.86** ±0.32	7.38* ±0.68	8.60** ±0.35	67.70** ±12.00	130.80** ±4.38	195.30** ±10.38	0.87* ±0.20	2.95** ±0.45	4.73** ±0.60	27.80** ±1.98	124.20** ±4.53	189.10* ±12.45
CV T-163												
0(Control)	4.70 ±0.35	10.57 ±1.00	13.04 ±1.50	72.30 ±5.46	152.50 ±10.46	178.60 ±12.31	2.25 ±0.30	8.44 ±1.50	11.59 ±1.85	61.20 ±5.35	258.20 ±10.46	360.50 ±15.56
1.70 × 10⁻⁴	6.91** ±0.65	12.89** ±1.15	18.41** ±2.50	95.80** ±8.35	183.50** ±12.45	257.90** ±15.46	3.76** ±0.30	11.43** ±1.25	18.72** ±2.00	111.70** ±7.35	326.60** ±20.35	503.10** ±25.15
8.52 × 10⁻⁴	4.07** ±0.30	7.21** ±0.95	9.35** ±1.08	57.30** ±4.65	78.00* ±9.35	128.50** ±11.35	1.83* ±0.25	4.10** ±1.00	9.71* ±1.08	44.86** ±5.00	109.20** ±15.44	308.20** ±20.44

**Significant at 1% level of significance.
*Significant at 5% level of significance.
±Values given are mean of 20 observations.

TABLE III

EFFECTS OF NICKEL PRETREATMENT TO SEEDS ON DRY WEIGHT CHANGES (mg $ORGAN^{-1}$) IN DARK GROWN SEEDLINGS OF *PISUM SATIVUM*

Seedling parts	Treatment (M Ni)								
	0 (Control)			1.70×10^4			8.52×10^4		
	Days after radicle emergence								
	3	5	7	3	5	7	3	5	7
				CV ARKEL					
Radicle	6.50 ±0.60	8.00 ±1.00	14.50 ±1.50	9.80** ±1.50	12.00** ±2.00	19.50** ±3.00	4.20** ±0.50	5.90** ±0.75	10.10** ±0.95
Epicotyl	5.50 ±0.50	10.40 ±1.50	19.50 ±2.00	8.40** ±2.00	14.90** ±2.25	25.00** ±3.00	3.50** ±0.35	8.20** ±0.95	15.00* ±2.00
Cotyledon	111.10 ±3.50	102.10 ±3.00	85.00 ±4.00	102.20** ±3.50	91.70** ±4.15	71.50** ±3.75	117.70** ±2.50	108.20** ±2.75	97.20** ±4.50
				CV BONNEVILLE					
Radicle	6.70 ±0.50	14.10 ±2.00	23.20 ±3.50	9.90** ±1.50	17.40* ±2.25	27.70** ±2.50	4.50** ±0.65	10.60** ±2.00	18.30** ±2.35
Epicotyl	5.00 ±0.65	13.90 ±1.00	21.30 ±2.50	7.30* ±1.00	16.10** ±1.50	24.80** ±2.00	4.20** ±0.50	10.00** ±0.95	15.70** ±1.50
Cotyledon	162.00 ±8.57	142.00 ±11.07	123.00 ±9.89	150.80** ±9.17	132.00** ±10.15	110.80** ±6.56	173.30** ±10.35	165.70** ±6.15	152.30** ±4.56
				CV T-163					
Radicle	7.30 ±0.56	11.60 ±1.00	15.60 ±1.85	10.50** ±1.00	14.60** ±1.75	19.70** ±2.00	5.40** ±0.46	8.10** ±0.95	12.40** ±1.50
Epicotyl	5.90 ±0.50	19.70 ±3.00	25.10 ±3.50	10.70** ±2.50	24.50** ±3.15	30.60** ±2.85	4.70** ±0.50	12.40** ±2.50	19.70* ±3.00
Cotyledon	235.50 ±6.97	215.20 ±7.15	203.50 ±8.47	224.80** ±5.00	206.50** ±4.75	191.10** ±3.95	244.60** ±6.00	228.10** ±5.00	213.60** ±4.50

**Significant at 1% level of significance.
*Significant at 5% level of significance.
±Values given are mean of 20 observations.

TABLE IV

EFFECTS OF NICKEL PRETREATMENT TO SEEDS ON TOTAL NITROGEN (N) AND PHOSPHATE (P) TRANSFER FROM COTYLEDON TO SEEDLING PARTS IN DARK GROWN SEEDLINGS OF *Pisum sativum* CULTIVARS

	CV ARKEL					CV BONNEVILLE					CV T-163				
Nickel Treatment, M	Days after radicle emergence 3	5	7	Increase from 3 to 7 day	Loss from 3-7 day	Days after radicle emergence 3	5	7	Increase from 3-7 day	Loss from 3-7 day	Days after radicle emergence 3	5	7	Increase from 3-7 day	Loss from 3-7 day
						Total N, mg organ^{-1}									
0 (Control)															
Radicle	0.326	0.390	0.656	0.330	—	0.357	0.726	1.096	0.739	—	0.462	0.714	0.976	0.514	—
Epicotyl	0.325	0.596	1.075	0.750	—	0.351	0.876	1.200	0.849	—	0.454	1.450	1.770	1.316	—
Cotyledon	6.115	5.325	4.314	—	1.801	8.165	6.759	6.060	—	2.105	11.398	9.189	8.009	—	3.389
1.70×10^{-4}															
Radicle	0.443	0.512	0.780	0.337	—	0.407	0.787	1.181	0.774	—	0.591	0.778	1.123	0.529	—
Epicotyl	0.469	0.792	1.269	0.800	—	0.424	0.894	1.316	0.892	—	0.537	1.624	1.962	1.425	—
Cotyledon	5.055	4.342	3.235	—	1.820	7.223	5.953	4.687	—	2.536	10.644	8.260	7.023	—	3.621
8.52×10^{-4}															
Radicle	0.240	0.329	0.537	0.297	—	0.278	0.618	0.919	0.641	—	0.386	0.551	0.811	0.425	—
Epicotyl	0.241	0.535	0.940	0.699	—	0.294	0.680	1.036	0.742	—	0.387	0.999	1.513	1.126	—
Cotyledon	6.868	6.088	5.263	—	1.605	9.749	8.732	7.767	—	1.982	12.376	11.063	9.184	—	3.192
						Total P, mg organ^{-1}									
0 (Control)															
Radicle	0.117	0.128	0.189	0.072	—	0.111	0.197	0.232	0.121	—	0.161	0.197	0.218	0.057	—
Epicotyl	0.110	0.177	0.234	0.124	—	0.095	0.195	0.257	0.162	—	0.136	0.296	0.326	0.190	—
Cotyledon	0.667	0.551	0.408	—	0.259	0.972	0.767	0.615	—	0.357	1.178	0.861	0.753	—	0.425
1.70×10^{-4}															
Radicle	0.137	0.150	0.225	0.088	—	0.139	0.209	0.277	0.138	—	0.178	0.219	0.256	0.078	—
Epicotyl	0.134	0.209	0.280	0.146	—	0.110	0.209	0.298	0.188	—	0.153	0.319	0.367	0.214	—
Cotyledon	0.591	0.440	0.315	—	0.276	0.814	0.594	0.434	—	0.380	1.052	0.764	0.609	—	0.443
8.52×10^{-4}															
Radicle	0.084	0.100	0.141	0.057	—	0.080	0.148	0.180	0.100	—	0.157	0.178	0.186	0.029	—
Epicotyl	0.088	0.172	0.200	0.112	—	0.084	0.160	0.220	0.136	—	0.118	0.248	0.296	0.178	—
Cotyledon	0.800	0.660	0.564	—	0.236	1.178	0.994	0.853	—	0.325	1.196	1.027	0.854	—	0.342

tyl and a related decrease in cotyledons as compared to controls. At 8.52×10^{-4} M Ni concentration, there is marked decrease in N content of radicle and epicotyl and a parallel decrease in its mobilization from the cotyledons. This is indicative of possible promotion of mobilization of nitrogen at 1.70×10^{-4} M concentration and supression of mobilization of N at 8.52×10^{-4} M, Ni.

Total P also exhibits the same pattern of mobilization as total N does (Table IV) In treated sets, the pattern of mobilization remains same as of control but the extents are influenced by nickel. At 1.70×10^{-4} M Ni concentration, P content of radicle and epicotyl increases more whereas, P content of cotyledons shows enhanced decrease with the age of seedlings as compared to controls. At 8.52×10^{-4}M, Ni concentration, the rise in P content of radicle and epicotyl is lesser as compared to control and the P level of cotyledon shows lesser decline with the age of seedlings. This again seems to indicate that nickel causes a promotion of P mobilization at lower concentration and supression of the same at higher concentration.

The above studies indicate again the occurrence of dose related organ specific and cultivar specific differences in response by Nickel. The twin effect of promotion of germination and seedling growth (Chaudhuri and Bhatnagar, 1952 Kariev, 1969 ; Tansykbaeva and Polyanichki, 1970 ; Buczek and Konarzewaki, 1968) and also inhibition of the same growth phenomenon (Lisanti, 1958 ; Agarwala *et al.*, 1961 ; Kusaka *et al.*, 1971) have been reported also by other workers. Effects of Ni on accumulation of several mineral elements have also been demonstrated by other workers (Vergnano, 1953 ; Crooke *et al.*, 1954 , Vergnano Gambi *et al.*, 1972). As heavy metals bind with proteins (Jerome and Ferguson, 1972), the duel effects of Ni on seedling growth, N and P mobilization could be through effect on enzymes crucial for germinaton and initial stages of seedling growth. Promotion and inhibition of protease activity by low and high concentrations respectively of cadmium had already been observed (Jain, 1980). These studies indicate that germination and seedling growth may be used for scanning of resistant susceptible cultivars.

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