EFFECT OF $CdCl_2$, NaF AND 2,4-DNP ON SOME METABOLIC CHANGES IN SOME CROP PLANTS

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SUMMARY: Water culture technique was used to study the role played by $CdCI_2$, NaF and 2,4-DNP when supplied in various levels on photosynthetic pigments, carbohydrate and nitrogen content in maize, sunflower and broad bean plants. The piphasic action of the test inhibitors involving stimulation of pigments biosynthesis at low concentrations and inhibition at higher concentrations was exhibited in case of maize and bean plants, while in case of sunflower this biphasic action was demonstrated only under the effect of 2,4-DNP. Accumulation of total carbohydrates and total nitrogen was substantially affected by the inhibitor supply. There was some evidence to indicate the presence of certain effective levels of the applied inhibitors manifesting depressive or promotive effects on the accumulation of the total carbohydrates and total nitrogen in the different organs of the treated plants.

Key Words: $CdCI_2$, NaF, 2,4-DNP;Pigment content, Carbohydrate, Zea mays, Helianthus annuus, Vicia faba.

INTRODUCTION

The effect of heavy metals upon various aspects of the environment is one of the major objective of research, Although a variety of heavy metals appear to be biologically essential in low concentrations, they are toxic at high levels and produce undesirable effects. Thus, the phytotoxicity of these metals has attained special concern to understand their role on plants and consequently on human health via the food web (Mahaffey *et al.*, 1975).

The pigment contents which could be regarded as a criterion for the photosynthetic activity (Sestak, 1967 and Raafat *et al.*, 1971), was found to be affected by cadmium treatments (Haghiri, 1973; Roat *et al.*, 1975; Malone *et al*, 1978; Markham *et al.*, 1980 a, b; Porter and Sheridan, 1981, Shahnaz, 1981). Concerning the effect of Cd on photosynthetic activity and carbohydrate accumulation, it was found that cadmium-treated plants exhibit reduced growth rate of shoot and root coupled with a pronounced reduction in photosynthetic activity and reduced carbohydrate accumulation (Huang *et al.*, 1974 and Shahnaz 1981).

However, the degree of inhibition may depend upon the plant type as well as the concentration of the inhibitors used.

Thus, this work was conducted to study in long-duration experiments the role played by three metabolic inhibitors ($CdCl_2$, NaF and 2,4-DNP) at certain concentrations, on photosynthetic pigments, carbohydrates and nitrogen accumulation in the different organs of maize, sunflower and broad bean plants.

MATERIALS AND METHODS

Maize (*Zea mays*), sunflower (*Helianthus annuus*) and broad bean (*Vicia faba*) plants were used in this investigation. Five-day old seedlings were selected for uniformity and transferred for two weeks to freshly prepared 1/2 strength Pfefer's nutrient solution. Micronutrient were supplied to the nutrient solution at concentrations similar to those used by Arnon and Hoagland (1940). The pH value of the nutrient solution was 5.7 ± 0.3. One-month old plants were treated with different concentrations of the applied metabolic inhibitor. This was carried out by exposing the plant roots, for 10 days, to nutrient solutions containing $CdCl_2$, NaF and 2,4-DNP at concentrations of 10^{-4}, 10^{-4}, 10^{-3} and 10^{-2} M. All the solutions experimented with were renewed every three days. Each treatment was carried out in three replicates.

At the end of the experimental period (10 days), the plant materials (roots, stems and leaves) were fixed at 105°C, then dried and analyzed to find out the changes in total carbohydrates and total nitrogen.

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For the determination of carbohydrate content, the anthrone sulphuric acid method was used (Fales, 1951 and Schlegel 1965). Nitrogen contents was also determined according to Paech and Tracey (1956).

The contents of chlorophyll a, b and carotenoids were determined according to Metzner *et al.* (1965) using the spectrophotometric method.

RESULTS AND DISCUSSION

The concentration of the photosynthetically active pigments (chlorophyll a, chlorophyll b and carotenoids) in the test plants is presented in Tables (1-3). These results clearly demonstrate a differential response of the three test plants to the applied inhibitors. The degree of this response depends mainly on the type of the inhibitor used and on the duration of the experiment as well as on test plant. A noticeable sensitivity in pigments biosynthesis in maize plants was displayed in the presence of high and low levels of the applied inhibitors. Generally, with the decrease of the inhibitor supply, in short-duration experiments, the inhibition of the pigments biosynthesis in maize leaves was partially or completely eliminated and further on it was reversed into promotion. In this context, there are two interesting observations. First, the stimulation effect of $CdCl_2$ on the total pigments accumulation occurred at 10^{-4}, 10^{-4} and 10^{-3} M while in case of NaF or 2,4-DNP it was exhibited at 10^{-5} and 10^{-4} M, thereafter any progressive increase in the inhibitor supply would be sufficient to account for the decrease in the total pigments concentration. Second, in short-duration experiments, the inhibition effect on the total pigments concentration of maize leaves occurred in the following order:

2,4-DNP > $CdCl_2$ > NaF

while in long-duration experiments it occurred in a different order:

$CdCl_2$ > 2,4-DNP > NaF

The biphasic action of the test inhibitors involving stimulation of pigments biosynthesis at low concentrations and inhibition at high concentrations was also exhibited by the treated bean plants.

Table 1: The concentration of pigments in sunflower leaves as affected by $CdCl_2$, NaF and 2,4-DNP supply in experiment of one day and 7-day period. The values given as mg/g fr. wt.

Treatment	One day period					7 day period			
		Chl.a	Chl.b	Carot.	Total	Chl.a	Chl.b	Carot.	Total
$CdCl_2$	0	1.282	0.353	0.405	2.040	0.773	0.375	0.391	1.539
	10^{-5}M	1.202	0.359	0.400	1.961	0.328**	0.246**	0.271**	0.839**
	10^{-4}M	1.181	0.369	0.391	1.941	0.285**	0.235**	0.284**	0.804**
	10^{-3}M	1.161	0.321*	0.400	1.882	0.342**	0.283**	0.315*	0.940**
	10^{-2}M	1.141	0.341	0.360**	1.842	0.304	0.267**	0.279**	0.850**
	L.S.D. at 5 %	0.164	0.028	0.023	0.240	0.044	0.024	0.070	0.120
	L.S.D. at 1 %	0.232	0.040	0.033	0.342	0.062	0.036	0.104	0.170
NaF	0	1.282	0.353	0.405	2.040	0.773	0.375	0.391	1.539
	10^{-5}M	1.080**	0.353	0.375**	1.808**	0.356**	0.243**	0.212**	0.811**
	10^{-4}M	1.090**	0.228**	0.364**	1.682**	0.447*	0.372	0.269**	1.088**
	10^{-3}M	0.960**	0.253**	0.388	1.601**	0.360**	0.325**	0.236**	1.021**
	10^{-2}M	0.890**	0.376	0.350**	1.616**	0.278**	0.253**	0.268**	0.799**
	L.S.D. at 5 %	0.126	0.042	0.018	0.046	0.270	0.032	0.070	0.110
	L.S.D. at 1 %	0.178	0.060	0.026	0.066	0.380	0.046	0.090	0.156
2,4-DNP	0	1.282	0.353	0.405	2.040	0.773	0.375	0.391	1.539
	10^{-5}M	1.248*	0.645**	0.387	2.280**	0.561**	0.372	0.252**	1.185**
	10^{-4}M	1.039**	0.619**	0.478**	2.136**	0.425**	0.295**	0.222**	0.942**
	10^{-3}M	0.946**	0.338	0.339**	1.623**	0.077**	0.032**	0.019**	0.128**
	10^{-2}M	0.926**	0.386	0.383	1.695**	0.035**	0.022**	0.079**	0.136**
	L.S.D. at 5 %	0.030	0.064	0.026	0.058	0.084	0.044	0.038	0.160
	L.S.D. at 1 %	0.064	0.090	0.038	0.082	0.120	0.062	0.058	0.230

* Significant differences.

** Highly significant differences as compared with control.

Table 2: The concentration of pigments in bean leaves as affected by $CdCl_2$, NaF and 2,4-DNP supply in experiment of one day and 7 day. The values given as mg/g fr. wt.

Treatment	One day period					7 day period			
		Chl.a	Chl.b	Carot.	Total	Chl.a	Chl.b	Carot.	Total
$CdCl_2$	0	0.862	0.126	0.404	1.392	0.770	0.246	0.266	1.282
	10^{-5}M	0.907	0.175**	0.405	1.487**	0.583**	0.364**	0.293	1.240
	10^{-4}M	0.891	0.175**	0.389*	1.437	0.613*	0.258	0.261	1.132**
	10^{-3}M	0.888	0.135	0.416	1.439	0.283*	0.156**	0.284	0.723**
	10^{-2}M	0.750**	0.100**	0.300**	1.150**	***	***	***	***
	L.S.D. at 5 %	0.048	0.018	0.015	0.048	0.110	0.023	0.028	0.061
	L.S.D. at 1 %	0.068	0.028	0.022	0.069	0.158	0.032	0.041	0.086
NaF	0	0.862	0.126	0.404	1.392	0.770	0.246	0.266	1.282
	10^{-5}M	0.926*	0.231**	0.392	1.549**	0.875	0.325*	0.293	1.493**
	10^{-4}M	0.866	0.239**	0.313**	1.418	0.714	0.248	0.262	1.224
	10^{-3}M	0.871	0.132	0.355**	1.358	0.593**	0.204	0.235	1.032**
	10^{-2}M	0.807*	0.124	0.300**	1.231**	0.543**	0.208	0.211	0.962**
	L.S.D. at 5 %	0.050	0.044	0.035	0.039	0.120	0.070	0.074	0.144
	L.S.D. at 1 %	0.072	0.062	0.049	0.056	0.170	0.100	0.105	0.204
2,4-DNP	0	0.862	0.126	0.404	1.392	0.770	0.246	0.266	1.282
	10^{-5}M	1.126**	0.261**	0.489**	1.876**	0.863**	0.297**	0.299	1.459**
	10^{-4}M	1.137**	0.190**	0.469**	1.796**	0.680**	0.245	0.214	1.139**
	10^{-3}M	0.833	0.122**	0.356**	1.311	0.309**	0.115**	0.107**	0.531**
	10^{-2}M	0.355**	0.063**	0.204**	0.622**	0.015**	0.010**	0.072**	0.097**
	L.S.D. at 5 %	0.160	0.028	0.030	0.208	0.028	0.028	0.058	0.029
	L.S.D. at 1 %	0.230	0.040	0.043	0.296	0.042	0.039	0.083	0.042

* Significant differences.

** Highly significant differences as compared with control.

*** Plants exhibited distorted appearance and the leaves withered and died by the end of the experimental period.

In case of sunflower plants this piphasic action was clearly demonstrated under the effect 2,4-DNP. At the different $CdCl_2$ or NaF levels the biosynthesis of pigments was profoundly retarded. This inhibition was more pronounced at moderate and high concentrations in case of the long duration experiments.

The inhibition of pigments biosynthesis in the test plants at certain concentrations of cadmium chloride, is in accordance with the results obtained by some other investigators using various plants. In this respect, treatment of *Ulva lactuca* and *Laminaria saccharina* with various concentrations of Cd (Markham *et al.*, 1980 a, b) reduced sharply their pigment content these authors stated that, in the affected organisms Cd pollution and intoxication cause a syndrome of effects rather than a single physiological deficiency. Also, one of the commonly reported symptoms of cadmium toxicity in higher plants is the decrease in chlorophyll content of their leaves (Marsh *et al.*, 1965; Haghiri, 1973; Root *et al.*, 1975; Del-Rio *et al.* 1978; Malone *et al.*, 1978; Porter and Sheridan, 1981 and Shahnaz, 1981). Haghiri (1973) mentioned that symptoms of cadmium toxicity resembled those of iron deficiency or chlorosis, where chlorophyll content severely dropped to very low levels.

It might seem paradoxical for an inhibitor to stimulate some particular reaction or phase of metabolic network but actually such stimulation was exhibited in the present work with respect to the biosynthesis of the photosynthetically active pigments which was stimulated at low concentrations of the applied inhibitors. There is a considerable reason to believe that the greater the number of interrelated metabolic phases in a living system, and the greater the complexity and organization of these phases, the more likely will it be that stimulation can occur. Various types of stimulation induced by metabolic inhibitors were revealed in other investigations.

Table 3: The concentration of pigments in maize leaves as affected by $CdCl_2$, NaF and 2,4-DNP supply in experiment of one day and 7 day period. The values are given as mg/g fr. wt.

Treatment	One day period					7 day period			
		Chl.a	Chl.b	Carot.	Total	Chl.a	Chl.b	Carot.	Total
$CdCl_2$	0	0.580	0.169	0.203	0.952	1.236	0.431	0.452	2.119
	10^{-5}M	0.720**	0.260**	0.266**	1.246**	1.199	0.414*	0.396**	2.009
	10^{-4}M	0.690**	0.222**	0.251**	1.163**	0.756**	0.256**	0.144**	1.156**
	10^{-3}M	0.590	0.213**	0.280**	1.083**	0.583**	0.224**	0.076**	0.883**
	10^{-2}M	0.450**	0.158	0.153**	0.761**	0.266**	0.056**	0.057**	0.379**
	L.S.D. at 5 %	0.034	0.016	0.025	0.042	0.046	0.015	0.027	0.122
	L.S.D. at 1 %	0.048	0.023	0.036	0.059	0.065	0.021	0.039	0.173
NaF	0	0.580	0.169	0.203	0.952	1.236	0.431	0.452	2.199
	10^{-5}M	0.726**	0.260**	0.258**	1.244**	1.412**	0.461	0.513**	2.386**
	10^{-4}M	0.709**	0.259**	0.217	1.185**	1.141*	0.489**	0.577**	2.207
	10^{-3}M	0.532*	0.252**	0.167**	0.951	1.665**	0.641**	0.456	2.762**
	10^{-2}M	0.526*	0.197*	0.139**	0.862*	0.885**	0.377*	0.371**	1.633**
	L.S.D. at 5 %	0.039	0.026	0.015	0.087	0.077	0.044	0.021	0.171
	L.S.D. at 1 %	0.057	0.037	0.021	0.124	0.109	0.063	0.030	0.243
2,4-DNP	0	0.580	0.169	0.203	0.952	1.236	0.431	0.452	2.119
	10^{-5}M	0.725**	0.296**	0.234**	1.255**	1.245	0.427	0.352**	2.024
	10^{-4}M	0.723**	0.220**	0.244**	1.187**	1.081**	0.407	0.374**	1.862**
	10^{-3}M	0.553	0.163	0.220**	0.936	0.686**	0.319*	0.242**	1.247**
	10^{-2}M	0.219**	0.055**	0.099**	0.373**	0.355**	0.197**	0.159**	0.691**
	L.S.D. at 5 %	0.036	0.015	0.012	0.089	0.066	0.082	0.022	0.134
	L.S.D. at 1 %	0.045	0.021	0.017	0.127	0.094	0.116	0.032	0.191

* Significant differences.

** Highly significant differences as compared with control.

Webb (1963) referred to the biphasic action of fluoride and azide on the endogenous respiration of yeast. In the case of fluoride a stimulation greater than 100% may occur at 20mM while at 30 mM a 70% inhibition takes its place. In addition the same author mentioned a few randomly chosen examples of various types of stimulation brough about by enzyme inhibitors such as arsenite, some of the quinones, phenylmercuric compounds and iodoacetate, which are perhaps sufficient to indicate the widespread occurrence of this phenomenon and he was led to conclude that the designation of a substance as an inhibitor must not prevent us from considering the substance as capable of a great variety of actions on a system so complex as the living cell.

The carbohydrate production in the different organs of the test plants was appreciably affected by the inhibitor supply. The results of Table 4 reveal that at all $CdCl_2$, NaF or DNP levels, the three plant organs (roots, stems and leaves) of the test plants generally showed a decrease in the total corbohydrate accumulation except in roots of bean plants grown at 10^{-5} M 2,4-DNP, where the carbohydrate production was raised over the control. The lowest carbohydrate production in the three organs of the three test plants was consistently found in plants grown at the highest inhibitor level. The least accumulation on of carbohydrates was estimated at 10^{-2} M NaF in sunflower leaves.

It is worth mentioning that the retarding effect of any of the investigated inhibitors on the production of total carbohydrates in the different organs of the test plants was partially alleviated at the lower levels of the inhibitors.

This inhibitory effect add more support to the results obtained by some other authors using various plant materials. (Younis *et al.*, 1971; Huang *et al.*, 1974; Shahnaz, 1981). The reduction in carbohydrate accumulation was attributed to the inhibited photosynthetic activity and the induces gross disturbances in carbohydrate metabolism.

The pattern of changes in the total nitrogen were different in the different plants and organs at the different levels of the inhibitors used. The inhibitory effects of

Table 4: The effect of various concentrations of $CdCl_2$, NaF and 2,4-DNP on the accumulation of total carbohydrates (T.C) in the different organs of sunflower, bean and maize plants. Data expressed as mg/g dry weight.

Treatments		Sunflower			Bean			Maize		
		Roots T.C	Stems T.C	Leaves T.C	Roots T.C	Stems T.C	Leaves T.C	Roots T.C	Stems T.C	Leaves T.C
$CdCl_2$	0	112.32	153.17	92.07	55.40	95.34	34.00	158.38	255.34	90.08
	10^{-5}M	94.16**	130.54**	72.04**	43.72**	70.67**	26.32**	137.61**	219.21**	67.28**
	10^{-4}M	86.49**	136.06**	65.34**	23.14**	67.34**	16.22**	125.61**	191.37**	64.77**
	10^{-3}M	57.04**	92.74**	60.42**	22.70**	66.99**	13.77**	78.31**	170.96**	46.80**
	10^{-2}M	44.52**	80.28**	52.43**	***	***	***	***	***	***
	L.S.D. at 5 %	2.54	1.53	1.01	1.57	0.96	0.37	3.59	2.27	0.95
	L.S.D. at 1 %	3.62	2.18	1.40	2.23	1.39	0.53	5.11	3.23	1.35
NaF	10^{-5}M	86.58**	130.84**	56.99**	39.90**	63.34**	26.83**	137.52**	216.99**	94.25**
	10^{-4}M	77.29**	123.18**	40.92**	38.55**	51.00**	22.68**	114.06**	207.22**	71.60**
	10^{-3}M	66.84**	97.17**	22.27**	25.04**	47.67**	18.91**	93.97**	204.40**	57.82**
	10^{-2}M	53.15**	75.43**	11.66**	21.62**	42.66**	15.45**	76.39**	158.78**	43.38**
	L.S.D. at 5 %	2.34	1.55	1.37	0.91	0.80	0.27	2.99	1.77	1.75
	L.S.D. at 1 %	3.32	2.19	1.78	1.29	1.14	0.39	4.25	2.51	2.99
2,4-DNP	10^{-5}M	113.62	114.88**	77.89**	62.06**	42.34**	23.00**	116.54**	203.70**	79.50**
	10^{-4}M	56.48**	94.88**	49.24**	57.20*	39.32**	21.04**	99.48**	190.70**	62.47**
	10^{-3}M	42.16**	76.17**	38.98**	31.07**	37.99**	8.93**	82.89**	138.08**	54.05**
	10^{-2}M	41.00**	65.63**	37.06**	***	***	***	***	***	***
	L.S.D. at 5 %	2.34	1.55	1.48	1.45	0.74	0.25	2.78	2.10	2.19
	L.S.D. at 1 %	3.33	2.19	2.53	2.06	1.05	0.36	3.95	2.99	3.11

* Significant differences.

** Highly significant differences as compared with control.

*** Plants exhibited distorted appearance and the leaves withered and died by the end of the experimental period.

Table 5: The effect of various concentrations of $CdCl_2$, NaF and 2,4-DNP on the accumulation of total Nitrogen (T.N) in the different organs of sunflower, bean and maize plants. Data expressed as mg/g dry weight.

Treatments		Sunflower			Bean			Maize		
		Roots T.N	Stems T.N	Leaves T.N	Roots T.N	Stems T.N	Leaves T.N	Roots T.N	Stems T.N	Leaves T.N
$CdCl_2$	0	25.13	35.55	31.39	65.81	87.30	45.95	166.66	53.34	49.99
	10^{-5}M	20.00	25.63**	28.38	58.85**	78.70**	41.03**	213.34**	52.83	46.66
	10^{-4}M	19.57**	22.49**	26.98*	45.51**	57.63**	29.57**	190.00**	70.47**	42.84*
	10^{-3}M	16.07**	19.33**	21.79**	41.95**	38.49**	21.33**	174.33**	69.53**	28.67**
	10^{-2}M	12.13**	13.81**	15.07**	***	***	***	***	***	***
	L.S.D. at 5 %	1.65	1.99	4.39	0.0180	1.67	0.55	5.57	2.58	6.23
	L.S.D. at 1 %	2.34	2.83	6.29	0.026	2.37	0.78	7.92	3.66	8.85
NaF	10^{-5}M	15.99**	26.17**	22.89**	57.85**	91.99**	23.41**	83.04**	59.89**	43.34
	10^{-4}M	15.43**	23.32**	24.31**	46.75**	66.35**	26.16**	59.91**	56.67**	28.27**
	10^{-3}M	13.59**	18.30**	21.75**	40.28**	26.40**	19.59**	40.80**	40.01**	20.07**
	10^{-2}M	12.76**	15.33**	20.47**	37.16**	16.38**	11.08**	32.14**	29.99**	18.33**
	L.S.D. at 5 %	1.56	2.44	3.13	0.917	0.31	0.6	4.15	1.77	8.81
	L.S.D. at 1 %	2.21	3.47	4.45	1.307	0.45	0.85	5.90	2.51	15.52
2,4-DNP	10^{-5}M	12.20**	23.70**	25.52*	60.58**	81.85**	35.22**	263.33**	85.00**	57.91
	10^{-4}M	10.70**	21.33**	23.46**	57.94**	78.60**	31.80**	214.66**	79.99**	41.64
	10^{-3}M	10.14	14.43**	19.05**	49.63**	66.08**	27.90**	213.33**	63.27**	34.78**
	10^{-2}M	7.25**	10.30**	18.01**	***	***	***	***	***	***
	L.S.D. at 5 %	1.56	3.29	4.11	0.674	0.74	0.60	5.06	2.94	9.40
	L.S.D. at 1 %	2.22	4.65	5.85	0.969	1.05	0.91	7.19	4.17	13.37

* Significant differences.

** Highly significant differences as compared with control.

*** Plants exhibited distorted appearance and the leaves withered and died by the end of the experimental period.

$CdCl_2$, NaF and 2,4-DNP concentrations on the total nitrogen were manifested in the different organs of sunflower and bean plants. Contrary to expectation, however, $CdCl_2$ or 2,4-DNP treatments up to 10^{-3} M stimulated the accumulation of total nitrogen in roots and stems of maize plants. Moreover, while the biphasic action of 2,4-DNP was exhibited with respect to the total nitrogen accumulation in maize leaves, all $CdCl_2$ and NaF levels manifested inhibitory effects. It is clear from the evidence presented here that it is somewhat difficult to follow the gross disturbances in nitrogen metabolism of the inhibitor-treated plants. The decrease in the total nitrogen content at certain levels of a metabolic inhibitor can be partially attributed to a reduction in protein synthesis. This opinion is favoured by Kremer and Markham (1982) who studied the inhibitory effect of cadmium in brown alga *laminaria saccharina*, and concluded that cadmium inhibits one or several steps in protein biosynthesis and thus leads to enzyme deficiency.

It is now clear that the role played by $CdCl_2$, NaF and 2,4-DNP, when suplied in various levels on plant growth and metabolic activities is complicated. There are various responses of the test plants and their organs at various levels of inhibitors. The data presented in this work may high-light a small gap in understanding the biphasic action of metabolic inhibitors.

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