Crop Sciences

SEED GERMINATION TRANSPIRATION RATE, AND GROWTH CRITERIA AS AFFECTED BY VARIOUS CONCENTRATIONS OF $CdCl_2$, NaF AND 2,4-DNP

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SUMMARY: The effect of $CdCI_2$, NaF and 2,4-DNP, when supplied in various concentrations on seed germination, seedling growth, transpiration rate and growth criteria of Zea mays, Helinathus annuus and Vicia faba was studied. The germination of the treated seeds significantly dropped as the concentration of the applied inhibitors increased, however, low doses of the applied inhibitors stimulated the germination of maize grains. The radicle and plumule lengths were considerably reduced at all levels of the applied inhibitors. Low concentrations of the inhibitors used had nearly small effect, if any, on transpiration rate, while the high levels strongly inhibited transpiration rate. Growth criteria (leaf area, and dry matter gain) of the different organs of bean and sunflower plants were sharply reduced; more prominently at moderate and high doses of the applied inhibitors. A biphasic action on dry matter gain in the different organs of maize plants was exhibited by the test inhibitors.

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

INTRODUCTION

Metabolic inhibition presents some of the most challenging unsolved problems of biology. The mode of the toxic action of various metabolic inhibitors, in both plants and animals, is one of the remaining aspects in which it cannot yet be said that biologists have made a decisive conclusion. Nevertheless, during the past three decades there has been a steady advance in our knowledge of the toxic effects of metabolic inhibitors.

The phytotoxicity of cadmium on growth and dry matter production of a number of cultivated plants have been reported by some investigators (Dekock, 1956; Agarwala and Kumar, 1962; Schroeder and Vinton 1962; Perry and Erlanger, 1971; Luckey *et al.* 1975 and Shahnaz, 1980). However the information regarding the effect of NaF or 2,4-DNP on growth and dry matter production of crop plants is meager.

Singh *et al.* (1978 a and b) observed that the various levels of fluorine as sodium fluoride significantly reduce the growth and the dry matter production of rice and wheat plants.

Also apart from extensive investigations, which have been concerned with toxic effects of metabolic inhibitors, relatively little work has hither to dealth with their effects on plant water relations. In this respect, Robert and William (1978) reported that various concentration of cadmium reduced transpiration of silver maple leaves. Also, impairment of stomatal function was reported by lonescu and Neamu (1973).

The aim of the present investigation is to give a further contribution to the effects of three metabolic inhibitors ($CdCl_2$, NaF and 2,4-DNP) on seed germination seedling growth, transpiration rate and growth criteria of three crop plants maize, sunflower and broad bean.

MATERIALS AND METHODS

Maize (Zea may), sunflower (Helianthus annuus) and broad bean (Vicia faba) plants were used in this investigation. The seeds obtained from Assiut University farm were throughly washed with distilled water and germinated on moist filter paper. Five-day old seedling were selected for uniformity and transferred for two weeks to Freshly prepared 1/2 strength Pfeffer'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 solution contain-

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ing $CdCl_2$, NaF and 2,4-DNP at concentrations of 10^{-5}, 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.

Germination were performed as described by Maftoun and Sepaskhah (1978), the percentage germination, and the lengths of radicle and plumule were measured after 5 days. Transpiration rate was measured during the period of treatment as described by Bozcuk (1975).

At the end of the experimental period the freshly harvested organs, Viz, roots, stems and leaves were weighed then dried in an aerated-oven at 70°C. Successive weighing was carried out until the constant dry weight of each sample was reached. The leaf area was measured by the disk method (Waston and Waston, 1935) and given as dm^2 $plant^{-1}$.

RESULT AND DISCUSSION

The data in Table 1 clearly shows that seed germination decreased as $CdCl_2$, NaF or 2,4-DNP concentration increased. The seed germination of the three experimental plants significantly dropped at the higher doses (10^{-1}, 10^{-2} M) of $CdCl_2$, while the highest dose (10^{-2} M) of either 2,4-DNP or NaF inhibited completely the germination of the treated seeds. Generally, it can be noticed that the inhibitory effects of NaF treatments on the germination of bean and sunflower seeds were more pronounced than in case of $CdCl_2$ or 2,4-DNP treatments. The germination of maize grains seemed to be less affected under the various concentrations of any of the three metabolic inhibitors used. Moreover, during four-day treatment period, low (10^{-5} M) and moderate (10^{-4} M) doses of NaF and low (10^{-5} M) concentration of $CdCl_2$ or 2,4-DNP resulted in relatively higher percentage of germination of maize grains than those of the control.

It appears probable from this unexpected response that the inhibitors used in this work are capable of acting as accelerators of the sequence of steps leading to germination and beginning long before the rupture of seed coat, if sufficiently lower concentrations were used. The low percentage germination of the treated seeds, especially at

Table 1: Effect of various concentrations of $CdCl_2$,NaF and 2,4-DNP on germination, length of radicle and plumule of sunflower, bean and maize plants.

Treatment		% germination			Length of Radicle			Length of plumule		
		Sunflower	Bean	Maize	Sunflower	Bean	Maize	Sunflower	Bean	Maize
$CdCl_2$	0	100.00	95.00	100.00	5.43	2.96	6.20	7.06	1.40	7.66
	10^{-5}M	86.65**	90.00	100.00	5.00	2.50*	6.10	6.50	1.07	7.33
	10^{-4}M	80.00**	83.30	86.65**	3.17**	2.23**	3.50**	3.50**	1.00	4.73*
	10^{-3}M	60.00**	76.65*	55.00**	2.40**	2.00**	0.66**	3.17**	0.76*	3.93**
	10^{-2}M	33.33**	50.00**	31.65**	0.50**	0.50**	0.33**	1.13**	0.10**	2.33**
	L.S.D. at 5%	3.80	13.50	5.99	0.65	0.46	1.55	0.64	0.49	2.11
	L.S.D.	5.43	19.20	8.52	0.92	0.65	2.21	0.90	0.69	2.99
NaF	10^{-5}M	80.00**	61.65**	100.00	3.00**	1.43**	6.33	3.66**	0.50**	3.50**
	10^{-4}M	61.65**	35.00**	100.00	1.43**	0.47**	5.66	1.93**	0.20**	5.17**
	10^{-3}M	16.65**	***	95.00**	0.73**	***	4.5*	1.17**	***	4.50**
	10^{-2}M	***	***	***	***	***	***	***	***	***
	L.S.D. at 5%	5.25	4.70	1.18	0.65	0.34	1.34	0.48	0.45	1.14
	L.S.D. at 1%	7.45	6.68	1.67	0.92	0.45	2.06	0.61	0.63	2.05
2, 4-DNP	10^{-5}M	85.00**	80.00*	100.00	3.33**	1.93**	5.80	3.00**	0.50**	6.00*
	10^{-4}M	76.65**	81.65	81.65**	1.83**	1.50**	5.17	2.70**	0.50**	3.17**
	10^{-3}M	25.00**	75.00*	53.30**	0.30**	1.50**	5.17	0.50**	0.50**	1.33**
	10^{-2}M	***	8.3**	***	***	0.50**	***	***	***	***
	L.S.D. at 5%	4.70	14.49	5.64	0.69	0.11	1.84	0.39	0.045	1.55
	L.S.D. at 1%	6.70	20.59	8.02	0.98	0.15	2.62	0.55	0.063	2.20

*Significant differences

** Highly significant differences, as compared with control.

*** Plants exhibited distored appearence and the leaves withered and died by the end of the experimental period.

the higher levels of the applied inhibitors, is comparable with the results of Shahnaz (1981). Who reported that cadmium considerably inhibits the germination of radish, lettuce, wheat and broom rice seeds.

The failure of the treated seeds to germinate at the high concentrations of the applied inhibitors may be consequence of retarded water uptake, inhibited cell divisions and enlargements in the embryo and or an overall decrease in metabolic activity relevant to these steps. The blockage of any one of the phases leading to germination may, and very likely will, completely inhibit the process of germination.

The radicle and plumule lengths of the treated seedlings (5 day old) of the test plants were considerably reduced at all levels of the applied inhibitors except at 10^{-5} M $CdCl_2$ where radicle and plumule lengths were more or less comparable with those of untreated seedlings. The adverse effects of the applied inhibitors on radicle and plumule growth were much more pronounced at the higher levels. Moreover, the inhibitory effect of a NaF and 2,4 DNF and 2,4-DNP treatments were generally more noticeable than those induced by $CdCl_2$ treatments (Table 1).

The inhibition of the embryonic root and plumule lengths of the germinated seeds, observed in this work at the high levels of the applied inhibitors, may be one aspect of the role of metabolic inhibitors in the overall phenomenon of plants growth. Evidence to support this suggestion may be obtained from the work Hesset *et al.* (1976), who reported a depressed root elongation in *Zea mays* seedlings in the presence of cadmium. A somewhat similar situation has been observed by Shahnaz (1981), who pointed out that during seed germination cadmium treatments affected reductions in root and shoot lengths. Further evidence to support the role played by cadmium in suspending root growth may be obtained from the hydroponic experiments of Malone et al. (1976). In which 100 mg CD/L was adequate to give 33% inhibition of lateral roots initiation of corn.

In the present study, the close correspondence between the inhibitory effects of NaF and 2,4-DNP, particularly, at 10^{-2} M, suggests that both NaF and 2,4-DNP are

Table 2: Changes in transpiration rate (q. H_2Odm^{-2} leaf area^{-1} day^{-1}) of sunflower bean and maize plants treated for 10-day period with different concentrations of $CdCl_2$, NaF and 2,4-DNP.

	Rate of transpiration		
Treatment	Sunflower	Bean	Maize
0	23.82	21.96	13.16
10^{-5}M	25.55	18.50**	10.88**
10^{-4}M	17.12**	9.21**	9.86**
10^{-3}M	9.66**	9.08**	9.70**
10^{-2}M	4.79**	5.93**	7.20**
L.S.D. at 5%	2.73	0.37	0.85
L.S.D. at 1%	3.88	0.52	1.21
10^{-5}M	26.54	22.97	12.74
10^{-4}M	17.46**	14.02**	11.72**
10^{-3}M	13.49**	14.24**	11.01**
10^{-2}M	7.82**	7.16**	8.58**
L.S.D. at 5%	3.23	0.55	0.86
L.S.D. at 1%	4.59	0.78	1.22
10^{-5}M	23.80	22.61**	13.28
10^{-4}M	18.54**	15.72**	7.88**
10^{-3}M	12.54**	6.57**	4.61**
10^{-2}M	6.77**	2.87**	2.00**
L.S.D. at 5%	1.29	0.41	1.68
L.S.D. at 1%	1.83	0.58	2.38

*Significant differences.

** Highly significant differences, as compared with control.

more or less equally effective in inhibiting the functional state of the germinating seeds, a prospect which deserves further consideration.

Transpiration rate: It was found that a concentration of 10^{-5} M of any of the inhibitors used had nearly small effect, if any, on the rate of transpiration of the test plants. Thereafter, a notable reduction was revealed in the presence of higher concentrations and the greatest inhibition was displayed at 10^{-2} M of any of the inhibitors used (Table 2).

Perhaps the most significant work concerning the role of the applied inhibitors on stomatal function has been done with cadmium due to its harmful effects on cellular activities.

In this respect, Bazzaz *et al.* (1974 a) demonstrated that interference with stomatal function is a primary mode of action of cadmium and several other heavy metals. The inhibitory effects of cadmium on transpiration reported here accord with those recorded by others using detached leaves or whole plants. In this context Robert and William (1978), reported that excised leaves of silver maple exposed to 0.045, 0.90 or 0.180 mM cadmium exhibited reduced transpiration. Bazzaz *et al.* (1974 b) observed very rapid reduction in transpiration of detached sunflower and corn leaves treated with various concentrations of cadmium ranging from 4.5 to 18 mM. Similar results were obtained using whole corn and sunflower plants (Carlson *et al.,* 1975). Actually, the inhibited transpiration should be considered harmful because, concomitantly, the potential gradient between leaves and roots which helps in water translocation should also be affected.

The results of this study and those obtained by others suggest that $CdCl_2$ and NaF inhibited transpiration by interference with stomatal function and/or by retarding water uptake by the test plants. Evidences to support this suggestion may be obtained from the results of various studies on the mode of their action. As determined by Radi (1974), cadmium chloride and sodium fluoride were found to strongly retard water uptake by sunflower plants.

Table 3: Effect of various concentrations of $CdCl_2$,NaF and 2,4-DNP on growth criteria of sunflower, bean and maize plants treated for 10-day.

Treatments		Leaf area dm^2 plant^{-1}			Dry matter (g plant^{-1})								
		Sunflower	Bean	Maize	Sunflower			Bean			Maize		
					(R)	(S)	(L)	(R)	(S)	(L)	(R)	(S)	(L)
$CdCl_2$	0	24.66	3.39	19.33	1.67	2.04	1.31	1.71	2.42	0.84	1.27	1.44	0.75
	10^{-5}M	19.00**	2.78*	16.66	0.97**	2.00	0.94*	1.24**	1.85**	0.70	1.30	1.50	0.75
	10^{-4}M	16.33**	1.21**	7.86**	0.85**	1.60	0.84**	1.33**	1.68**	0.22**	0.63	0.61**	0.44**
	10^{-3}M	13.83**	1.18**	5.00**	0.72**	1.44**	0.47**	1.05**	1.57**	0.20**	0.64	0.53	0.30**
	10^{-2}M	6.00**	-	-	0.51**	0.96**	0.35**	-	-	-	-	-	-
	L.S.D. at 5%	0.89	0.61	2.86	0.59	0.50	0.29	0.29	0.39	0.23	0.65	0.28	0.13
	L.S.D. at 1%	1.28	0.87	4.07	0.85	0.67	0.42	0.42	0.55	0.33	0.93	0.39	0.18
NaF	10^{-5}M	22.33**	3.64	17.33	1.05*	2.40	1.21	1.50	0.95**	0.95	1.57	1.86*	0.82
	10^{-4}M	17.00**	2.79*	14.66**	0.86**	1.30	0.97*	1.45	0.49**	0.49**	1.45	1.50	0.81
	10^{-3}M	14.66**	2.55**	9.66**	0.75**	1.37	0.83**	1.40*	0.41**	0.41**	1.49	0.75**	0.61
	10^{-2}M	11.00**	1.39**	7.50**	0.68**	1.63	0.52**	0.73**	0.29**	0.29**	0.91	0.59**	0.59
	L.S.D. at 5%	0.96	0.29	2.21	0.51	0.85	0.29	0.27	0.17	0.17	0.78	0.32	0.33
	L.S.D. at 1%	1.40	0.41	3.14	0.73	1.21	0.42	0.37	0.25	0.25	1.004	0.46	0.46
2, 4-DNP	10^{-5}M	18.00**	2.84**	11.00**	1.14	2.07	1.36	1.15**	0.71**	0.71	1.45	1.36	0.95**
	10^{-4}M	15.66**	2.35**	8.16**	0.84**	1.42	1.53	1.00**	0.48**	0.48	1.02	1.19	0.83
	10^{-3}M	12.66**	1.22**	5.10**	0.70**	0.50**	0.99*	1.00**	0.35**	0.35**	0.75	1.29	0.56**
	10^{-2}M	9.33**	-	-	0.48**	0.40**	0.69**	-	-	-	-	-	-
	L.S.D. at 5%	0.88	0.295	2.02	0.57	0.97	0.42	0.28	0.134	0.18	0.72	0.57	0.12
	L.S.D. at 1%	1.24	0.42	2.87	0.81	1.37	0.59	0.39	0.149	0.25	1.03	0.81	0.15

*Significant differences at compared with control.

** Highly significant differences, as compared with control.

(R) Roots, (S) Stems, (L) Leaves

The impairment of stomatal function reported here at certain concentrations of $CdCl_2$ or NaF could be attributed to retard water absorption. The latter conclusion cannot be applied to the results of the present work concerning the role played by 2,4-DNP, which in the light of Radi's findings, had nearly no effect on water uptake.

A possible mode of action of cadmium may be interference with diffusive resistance of leaves to carbon dioxide and water vapour transport (Robert and William, 1978). Also, cadmium which is known to affect a host of enzyme systems (Vallee and Ulmer, 1972) may alter stomatal function, indirectly, by affecting potassium ion pump controlling the movement of potassium between guard cells and adjacent subsidiary cells (Humble and Hisao, 1969 and 1970, Zelitch, 1971), or any other additional aspects including changes in chlorophyl and accessory pigment content of leaves (Hewitt 1954, Bazzaz and Govindjee 1974).

The leaf area tended to decrease with the increase of the inhibitor supply. In case of sunflower plants the greatest inhibition was displayed in the presence of 10^2M $CdCl_2$, while in case of maize and bean plants treated with 10^{-2}M $CdCl_2$ or 2,4-DNP the leaves withered and died. In this respect, sodium fluoride treatments exhibited comparatively the lowest inhibitory effects on leaf area in comparison with those of the other two inhibitors (Table 3).

The response of the test plants to the high levels of the applied inhibitors was reflected in decreased growth. The presence of any of the applied inhibitors in the culture media at a concentration of 10^{-3} or more greatly attenuated the dry matter gain in the different organs of the experimental plants (Table 3). The inhibitory effects of cadmium on the growth of the test plants add more support to the results obtained by some other authors using various plants (Hewitt, 1984; Dekock, 1956; Agarwala and Kumqr 1962; Schroeder and Vinton, 1962; Page *et al.*, 1972; Haghiri, 1974; Huang *et al.* 1974; Cunningham *et al.*, 1975; Dowdy and Ham, 1977; Bonaly *et al.*, 1978 and Markham *et al.*, 1980 a and b).

The inhibitory effects of cadmium on plant growth was also established by other investigators at relatively low levels. In this respect, Page *et al.* (1972), claimed that cadmium concentration of 40 μM reduced the growth of barley and at 1 μM for soybean huang *et al.* (1974); Cunningham *et al.*, (1975); working on soybean confirmed the inhibitory role of cadmium on the growth rate of roots, stems and pods at a concentration of 18 μM.

Moreover, the occurrence of a considerable reduction in dry matter yields of the test plants under the influence of high concentrations of sodium fluoride (Table 3), is in agreement, with the results of Singh *et al.* (1978a), who reported that, with increasing levels of F above 50 ppm, decreased the dry matter yield of rice plants (Oryza sativa) in the early growth stages and grain yield at maturity. The same authors (1978b), with wheat plants recorded that increased F uptake resulted in a significant reduction in the wheat yield. They also, noted that, though in early growth stages the harmful effect of fluorine was noticeable only at the highest level (200 ppm F as sodium fluoride) the grain yield was reduced significantly, even at 25 ppm applied fluorine. This again confirms the suggestion that the response of crop plants to metabolic inhibitors, at sublethal concentrations, is generally reflected in decreased growth and hence depressed productivity.

Retention of metabolic balance in plant tissues is considered to be an assurance for normal plant growth. In the light of this fact, the inhibited plant growth and depressed productivity could be attributed to metabolic disorders induced by the inhibitors.

It should be recalled that although all treatments involving inhibitors at high concentrations (10^{-3} - 10^{-2} M) strongly inhibited the whole matter gain, the effectiveness of the applied inhibitors varied in the different test plants.

a. with sunflower and bean plants inhibition effect occurred in the following order:

2,4-DNP > $CdCl_2$ > NaF.

b. with maize plants, the inhibition effect occurred in a different order:

$CdCl_2$ > 2,4-NDP > NaF.

On the other hand, the promotive effect of low concentrations of the applied inhibitors, on the growth of maize plants was observed in the present investigation. The stimulatory effect of NaF at 10^{-4} and 10^{-5} M on fresh and dry weight gain in the different organs of maize plants was also exhibited by $CdCl_2$ or 2,4-DNP at concentration of 10^{-5} M.

This inhibitor-stimulated growth of maize plants again confirms the suggestion that a metabolic inhibitor may exhibit a biphasic action involving stimulation at lower concentrations and inhibition at higher concentrations. The interpretation of this response and the factors that determine its magnitude are of great importance in inhibition studies.

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