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Growth, carbohydrates and associated invertase and amylase activities in castor bean and maize as affected by metribuzin and NaCl*

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Abstract

Growth parameters (leaf area, length of shoot and root, water content and dry matter accumulation), contents of reducing sugars and saccharose as well as activities of α- and β-amylases of castor bean and maize seedlings and adult plants supplemented with 0.5 $\mu g\ g^{-1}$ and 2.5 $\mu g\ g^{-1}$ of metribuzin either alone or in combination with 50 $\mu g\ g^{-1}$ NaCl, were increased significantly whereas at high concentrations (5 and 10 $\mu g\ g^{-1}$) of herbicide, an opposite response was apparent. On the other hand, polysaccharide content and invertase activity of castor bean and maize seedlings and adult plants were significantly decreased in response to low concentrations (0.5 and 2.5 $\mu g\ g^{-1}$) of metribuzin and increased significantly at high concentrations (5 and 10 $\mu g\ g^{-1}$) of the herbicide either alone or in combination with 50 $\mu g\ g^{-1}$ NaCl. Total carbohydrate contents of castor bean and maize seedlings and whole plants treated with herbicide either alone or in combination with NaCl did not change significantly. Growth parameters, carbohydrate fractions contents and activities of enzymes in both castor bean and maize seedlings and whole plants treated with herbicide alone were consistently higher than those values detected in plants treated with herbicide in combination with NaCl.

Introduction

The physiological effects of herbicides on plants are dependent on the herbicide used, plant species, tissue type, herbicide concentration and the duration of treatment. Metribuzin (4-amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazine-5-one) was reported as ineffective in pre-emergence treatment for large crabgrass control (Callahan 1986). At sublethal doses, Tóth and Belejová (1987) found that metribuzin inhibited growth of spring barley and winter wheat grown in sand culture.

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Also, Gabr *et al.* (1988 a, b) emergence observed that the herbicide on the recommended concentration of metribuzin to soybean, when applied as pre-emergence herbicide on the surface of soil, caused no significant effect on stem length, number of leaves, leaf area and number of internodes per plant.

Prensser *et al.* (1984) found that malathin applied to 21-d-old *Vicia faba* plants, reduced the soluble, and increased the insoluble sugar contents. On the other hand, metribuzin in higher concentrations decreased the saccharide contents, the decrease being rather larger in polysaccharides than in reducing sugars. The maximum inhibition of polysaccharides accumulation and water soluble sugars content was achieved in seedlings treated with the metribuzin concentration of 130×10^{-2} mM (Hamed 1990).

Several enzymes have been shown to be affected by herbicide treatments. The observed effects of herbicide on hydrolytic enzymes (α- and β-amylases, invertase and protease) were found to vary from one plant to another and appeared to be a function of the concentration used and duration of treatment (Wybieralski and Wybierlaska 1984, Gabr *et al.* 1988a, Hamed 1990).

Thus, the aim of this work was to investigate further metabolic responses of castor bean and maize seedlings and adult plants, to pre-planting culture incorporation with different concentrations of metribuzin either alone or in combination with 50 $\mu g\ g^{-1}$ NaCl.

Materials and methods

Time course experiment: Homogeneous seeds of castor bean (*Ricinus communis* L. cv. Baladi) and maize (*Zea mays* L. cv. Giza 2), were used. The procedures of sterilization of seeds, germination and growing of seedlings as well as the experimental set-up were the same as previously described by Steingrover (1983) and Hasaneen *et al.* (1989).

Samples for determination of growth parameters, carbohydrate fractions and activities of α- and β-amylase and invertase were taken of seedlings and adult plants after 3 and 21 d from transplantation. Leaf area was measured by square-paper method, fresh and dry mass after drying samples in an oven at 70 °C to constant mass.

Determination of saccharides: The direct reducing value equivalent to the amount of reducing sugars was determined after Nelson (Bell 1955). The total reducing value was estimated from absorbance at 700 nm after hydrolysis by invertase. The difference between the total and the direct reducing values was taken as the saccharose content. Polysaccharides were determined in the dry residue of alcohol extract of soluble sugars (Younis *et al.* 1969).

Determination of enzyme activities : Amylases were extracted by the method of Gibbs (1952). The activity of α-amylase was estimated by the method of Street and Close (1956) and that of β-amylase by the method of Lyda *et al.* (1970) as described by

Moustafa and El-Shafey (1978). Invertase was extracted and its activity determined according to Russell and Jimmy (1980) as described by Moustafa *et al.* (1981).

Results and discussion

Changes in growth parameters: Treatment of both castor bean and maize plants species with 0.5 μg g^{-1} and 2.5 μg g^{-1} of metribuzin either alone or in combination with 50 μg g^{-1} NaCl induced significant increase in the three growth parameters, *i.e.* leaf area, length of shoot and root measured (Table 1). On the other hand, 5 μg g^{-1} and 10 μg g^{-1} of metribuzin + 50 μg g^{-1} NaCl led to significant decrease in root and shoot length, in leaf area and in fresh and dry mass of both species. The changes were more pronounced in samples treated with herbicide alone than in those supplemented with herbicide in combination with NaCl.

In support of our results, Hamed (1990) stated that, under hydroponic culture conditions, the response of faba bean seedlings to metribuzin varied with the concentration and with the time of exposure to the herbicide. Stimulation of radicle elongation by 6.5×10^{-2} mM occurred after 3 and the same concentration of metribuzin induced a stimulation of shoot elongation during the early stages of growth. However, as the concentration of herbicide increased, there was a rapid increase in the inhibition of both radicle and shoot elongation.

Fedtke (1982) and Moreland *et al.* (1982) compiled a partial list of physiological processes considered to be most important during the germination and growth of seedlings as these processes may be related to possible action by a variety of herbicides. Among these processes are ion transport, membrane permeability, cell division and cytological behaviour of cells.

As consequence of herbicide and NaCl treatment, in the literature, inhibition of hormone regulation (Fedtke 1982a), protein synthesis (Duke *et al.* 1975) and pigmentation (Ensminger and Hess 1985) have often been observed. These responses are presumably due to a direct or indirect effect of herbicide and/or salinity on the activity of some enzyme systems. Furthermore, plant growth is primarily related to cell division and cell enlargement, and both processes are known to be controlled by plant growth regulators. Thus, the inhibition in growth of both species, either normal or stressed, as a result of herbicide application, appears to be correlated with hormonal biosynthesis in the affected plants.

Changes in carbohydrate content: Reducing sugars and saccharose contents in both species were significantly increased or decreased after treatment with metribuzin either alone or supplemented with NaCl in concentrations of 0.5 and 2.5 μg g^{-1}, or 5 and 10 μg g^{-1}, respectively. On the other hand, polysaccharide contents was significantly decreased at 0.5 μg g^{-1} and 2.5 μg g^{-1} metribuzin alone or supplemented with 50 μg g^{-1} NaCl. However, significant increases were maintained in polysaccharides in plants treated with 5 μg g^{-1} and 10 μg g^{-1} herbicide alone or combined with NaCl. Insignificant changes in total carbohydrate contents of both plants treated with different concentrations of herbicide either alone or in combination with NaCl were apparent. The changes were most pronounced with the herbicide alone (Table 2). In this connection, Hamed (1990) reported variable changes in the whole carbohydrate contents of *Vicia faba* treated with different concentrations of metribuzin in culture media. The lowest concentration of metribuzin seemed to be without effect on total soluble sugars, but of an inhibitory effect on polysaccharides accumulation.

Table 1. Effect of different concentrations of metribuzin alone or in combination with NaCl on growth parameters of castor bean and maize seedlings (7 d) or adult plants (25 d). * - $P = 0.05$, ** - $P = 0.01$.

Concentration		Seedlings				Adult plants				
Metribuzin [μg g^{-1}]	NaCl [μg g^{-1}]	Leaf area [cm^2]	Radicle length [cm]	Fresh mass [mg]	Dry mass [mg]	Leaf area [cm^2]	Shoot length [cm]	Root length [cm]	Fresh mass [mg]	Dry mass [mg]
Ricinus communis										
0	0	3.9	4.1	539	101	29.0	22.1	6.1	3159	788
0.5	0	8.6**	6.3**	742**	191**	57.1**	33.6**	9.2**	4005**	1087**
2.5	0	6.3**	5,1**	641**	182**	40.1**	30.4**	7.4**	3751**	1054**
5.0	0	2.5**	2.0**	401**	82**	21.5**	16.0**	4.0**	2603**	543**
10.0	0	2.1**	1.0**	230**	71**	16.3**	10.5**	3.0**	1950**	504**
L.S.D. at 5% level		0.19	0.20	26.5	5	1.45	1.10	0.30	157.5	39.0
L.S.D. at 1% level		0.29	0.30	39.7	7.5	2.17	1.65	0.45	236.2	58.5
0	50	2.1	1.4	316	53	14.1	8.0	3.5	2502	424
0.5	50	4.1**	2.5**	502**	91*	29.2**	14.1**	7.1**	3102**	651**
2.5	50	3.3**	2.8**	421**	77	19.0**	10.2**	5.6**	2801**	632**
5.0	50	1.8**	1.0**	253**	54	11.0**	3.2**	2.5**	1906**	321**
10.0	50	0.8**	0.5**	202**	40**	3.0**	2.2**	1.0**	1460**	302**
L.S.D. at 5% level		0.10	0.07	15.5	2.6	0.70	0.40	0.17	125.0	21.0
L.S.D. at 1% level		0.15	0.10	23.0	3.9	1.05	0.60	0.26	187.5	31.5
Zea mays										
0	0	2.3	2.9	700	162	33.6	17.1	6.3	350.2	842
0.5	0	6.3**	4.8**	1401**	291**	58.2**	30.2**	12.0**	445.1**	1058**
2.5	0	4.4**	3.6**	1150**	282**	42.1**	22.1**	9.1**	360.1	1002**
5.0	0	1.8**	2.0**	602**	121**	18.1**	10.2**	4.6**	260.1**	632**
10.0	0	1.0**	1.5**	452**	116**	10.2**	7.3**	2.3**	160.2**	611**
L.S.D. at 5% level		0.11	0.14	35.0	8.0	1.68	0.85	0.31	17.50	42
L.S.D. at 1% level		0.16	0.21	52.5	12.0	2.52	1.28	0.47	26.25	63
0	50	1.5	1.9	413	85	16.2	8.2	3.9	260.1	630
0.5	50	2.9**	2.4**	652**	110**	36.0**	13.1**	6.2**	340.6**	722**
2.5	50	2.0**	2.0**	502**	102**	24.2**	10.6**	5.0**	290.1**	700**
5.0	50	1.1**	1.1**	331**	63**	11.1**	6.0**	2.9**	170.2**	521**
10.0	50	0.6**	0.6**	223**	54**	7.1**	4.2**	1.4**	100.2**	500**
L.S.D. at 5% level		0.07	0.09	20.5	4.2	0.81	0.41	0.19	13.00	31.5
L.S.D. at 1% level		0.11	0.14	30.0	6.3	1.21	0.61	0.29	19.50	47.2

Table 2. Effect of different concentrations of metribuzin either alone or in combination with NaCl on carbohydrate content [mg(glucose) kg^{-1}(dry matter)] in castor bean and maize seedlings (7 d) and adult plants (25 d). * - $P = 0.5$, ** - $P = 0.1$.

Concentration metribuzin [μg g^{-1}]	NaCl [μg g^{-1}]	*Ricinus communis* Reducing sugars	Sucrose	Polysaccharides	Total saccharides	*Zea mays* Reducing sugars	Sucrose	Polysaccharides	Total saccharides
Seedlings:									
0	0	3 359	2 383	12 503	18 245	2 252	3 809	16 595	22 656
0.5	0	4 056**	3 132**	11 515*	18 703	3 053**	4 902**	15 135**	23 090
2.5	0	3 525	2 601**	11 409**	17 535	2 509**	4 207**	15 013**	21 729
5.0	0	2 882**	2 003**	14 812**	19 697	1 702**	3 218**	18 504**	23 424
10.0	0	2 135**	1 235**	15 659**	19 028	1 315**	2 565**	28 699**	32 579
L.S.D. at 5% level		167	119	625	912	112	190	829	1 132
L.S.D. at 1% level		251	178	937	1 368	168	285	1 244	1 698
0	50	2 643	1 441	17 048	21 132	1 128	2 834	22 132	26 094
0.5	50	3 209**	2 303**	14 355**	19 867	2 004**	3 523**	18 875**	24 422
2.5	50	2 834*	1 602*	15 285**	19 641	1 591	2 915	19 803**	24309
5.0	50	2 005**	1 005**	19 358**	22 368	1 003**	2 323**	23 853*	26 179
10.0	50	1 606**	537**	20 006**	22 149	773**	2 009**	24 039**	26 821
L.S.D. at 5% level		132	720	852	1 056	56	141	1 106	1 304
L.S.D. at 1% level		198	1 080	1 278	1 584	84	212	1 659	1 956
Adult plants:									
0	0	4 501	3 873	18 303	26 677	3 972	5 571	24 005	33 548
0.5	0	5 904**	4 898**	14 305**	25 107	5 015**	6 658**	19 601**	31 274
2.5	0	5 008**	4 202**	15 603**	24 813	4 574**	6 101**	20 876**	31 551
5.0	0	3 976**	3 293**	21 302**	28 571	3 139**	4 846**	27 513**	35 498
10.0	0	3 055**	2 408**	22 407**	27 870	2 391**	3 623**	28 497**	34 511
L.S.D. at 5% level		225	193	915	1 333	198	278	1 215	1 692
L.S.D. at 1% level		337	290	1 372	2 000	297	417	1 822	2 338
0	50	3 202	2 711	24 578	30 491	2 617	3 952	30 408	36 977
0.5	50	4 447**	3 599**	22 005**	30 051	3 504**	5 225**	26 613**	35 342
2.5	50	3 663**	2 956	22 721**	29 340	2 895*	4 308**	27 885**	36 088
5.0	50	2 742**	2 115**	27 814**	32 671	2 063**	3 331**	33 173**	38 567
10.0	50	2 305**	1 642**	28 502**	32 449	1 739**	2 974**	34 291**	39 004
L.S.D. at 5% level		160	135	1 228	1 524	130	197	1 520	1 848
L.S.D. at 1% level		240	203	1 842	2 286	195	296	2 280	2772

Prensser *et al.* (1984) found that malathin reduced the content of sugars and increased that of insoluble saccharide in *Vicia faba* plants.

The depression in polysaccharides that occurred concomitantly with the increase in reducing sugars and saccharose contents in castor bean and maize seedlings and in whole plants treated with low concentrations of metribuzin either alone or in combination with NaCl can be attributed to the rapid degradation of polysaccharides by hydrolytic enzymes and/or to the inhibition of photosynthesis. In this connection,

Table 3. Unit activity determinations of invertase, α- and β-amylase of castor bean and maize treated with different concentrations of metribuzin either alone or in combination with NaCl. The values listed are given as units per 1000 cm^3 enzyme preparation. * - $P = 0.05$, ** - $P = 0.01$.

Concentration		Seedlings			Adult plants		
Metribuzin [$\mu g\ g^{-1}$]	NaCl [$\mu g\ g^{-1}$]	Invertase	α-amylase	β-amylase	Invertase	α-amylase	β-amylase
Ricinus communis							
0	0	1112	8433	6748	653	7202	5690
0.5	0	742**	9188*	7595**	419**	8015**	6502**
2.5	0	894**	8723	7227*	496**	7546	6097**
5.0	0	1441**	7438**	5626**	1008**	6009**	4756**
10.0	0	1829**	6259**	4335**	1414**	5098**	3118**
L.S.D. at 5% level		55	421	337	32	36	284
L.S.D. at 1% level		83	632	505	48	54	426
0	50	1708	6715	4673	1233	5234	3434
0.5	50	1218**	8074**	6108**	735**	6536**	5107
2.5	50	1525**	7130	5116**	1189	5459	3945**
5.0	50	1938**	5828**	4000**	1587**	4000**	2703**
10.0	50	2150**	5404**	3879**	1663**	3624**	2251**
L.S.D. at 5% level		85	335	233	615	261	171
L.S.D. at 1% level		127	503	350	922	392	257
Zea may							
0	0	1545	8779	7907	1126	7434	6145
0.5	0	1193**	9873**	8635**	821**	8433**	6844**
2.5	0	1319**	9106**	8239	987**	7722	6439
5.0	0	1872**	7673**	6214**	1363**	6321**	5064**
10.0	0	2446**	6736**	5000**	1759**	5257**	4521**
L.S.D. at 5% level		77	438	395	56	371	307
L.S.D. at 1% level		115	657	592	84	557	460
0	50	2234	6801	5388	1625	5676	4000
0.5	50	1633**	8205**	6773**	1224**	4693**	5573**
2.5	50	2096	7328*	5626	1553	5919	4994**
5.0	50	2618**	6005**	4509**	2090**	4436**	3406**
10.0	50	2877**	5574**	4101**	2258**	3807**	3118**
L.S.D. at 5% level		111	340	269	81	283	200
L.S.D. at 1% level		167	510	403	121	425	300

Fedtke (1979) and Hamed (1990) rendered the low carbohydrate accumulation in soybean and faba bean plants to the decreased photosynthetic rate. Furthermore, Richard *et al.* (1983) observed the depression of photosynthesis in soybean leaves when s-triazine was applied.

On the other hand, the decrease in reducing sugars and saccharose that occurred concurrently with the increase in polysaccharide and in total saccharides can be explained by changes in enzyme activities and/or in net photosynthetic rate.

Changes in enzyme activities: In both species treated, α- and β-amylase activities were significantly increased in comparison with control. On the other hand, the activities of the two enzymes decreased significantly in seedlings and in adult plants treated with 5 and 10 μg g^{-1} metribuzin either alone or with NaCl. Again, the response was more pronounced in normal than in NaCl-stressed plants (Table 3). Also, the activity of α-amylase was higher than that of β-amylase in seedlings and adult plants treated. The activities of the two enzymes in seedlings was much higher than in adult plants.

On the other hand, 0.5 and 2.5 μg g^{-1} metribuzin either alone or supplemented with 50 μg g^{-1} NaCl led to significant decrease in invertase activity in both species tested. However, the addition of 5 and 10 μg g^{-1} herbicide alone or with NaCl into the Hoagland media induced significant increase in the invertase activity in both plants; the changes were more pronounced in combination with NaCl (Table 3).

In accordance with the present results, Hamed (1990) showed that the activities of α- and β-amylases and protease in faba bean seedlings treated with 6.5×10^{-2} mM metribuzin were generally above the control level during germination and growth. However, the relatively low concentration of metribuzin (13×10^{-2} mM) stimulated the activities of α- and β-amylases, but inhibited the activity of protease. Increasing the concentration of herbicide applied over 13×10^{-2} mM was concomitant with corresponding decreases in the biosynthesis of amylases and proteases. Such effects have also been reported by Klepper (1974) and Gabr *et al.* (1988).

Moustafa *et al.* (1981) reported slight stimulation of invertase activity in soybean treated with the herbicide diphenamide. This report is in agreement with Begonia *et al.* (1971) and Klepper (1974) who found enhancement of the activities of certain enzymes in response to relatively low doses of the herbicide. The changes in saccharose content under the low and high doses of the herbicide, either alone or in combination with NaCl, are parallel with the inverse changes in invertase activity (Tables 2 and 3).

The inhibitory effect of metribuzin alone or in combination with NaCl on α- and β-amylases and invertase activities could be attributed to the effects on protein synthesis and subsequently reduction or blockage of *de novo* protein necessary for enzymes and coenzymes. In fact, as a result of herbicidal treatment, the activity of several enzymes was found to be modified. α-amylase in germinating wheat and mung bean seeds (Dalvi *et al.* 1972) and other enzymes of carbon and nitrogen metabolism in pea and sweet maize leaves (Wu *et al.* 1971). On the other hand, some specific enzymes are enhanced as a result of herbicide action; especially s-triazine that stimulate some enzymes responsible for starch degradation (Scarponi *et al.* 1989).

The observed stimulation of α- and β-amylase and inhibition of invertase activities was perhaps the consequence of a generally increased rate of protein synthesis. Another explanation could be the possible effect of metribuzin and NaCl on prosthetic groups of the enzymes. Also, it seems possible that the herbicide caused intramolecular changes in protein sulphydryl group which could result in a modification of the enzyme structure (Scarponi *et al.* 1989, Nemat-Allah 1991).

Our results concerning the combined effect of herbicide and NaCl may be explained on the basis of the chemical stress that occurred in the plant tissues. The stressed plants exert a considerable amount of energy to counteract or to detoxify the herbicide and salt.

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