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Effects of metribuzin herbicide on nitrogen, pigments, protease and nitrate reductase activity of normal and NaCl-stressed castor bean and maize plants

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Abstract

Addition of 0.5 and 2.5 g m^{-3} of metribuzin into Hoagland nutrient media, either alone or in combination with NaCl, induced significant decreases in nitrate-, amino-, ammonia-, and total soluble-N contents, whereas significant increases in these nitrogen fractions were apparent in maize and castor bean seedlings and plants treated with high concentrations (5 and 10 g m^{-3}) of the herbicide, again either alone or in combination with NaCl. Protein- and total-N contents increased and decreased at low and high concentrations of the herbicide, respectively. The contents of chlorophylls *a* and *b*, as well as carotenoids of both castor bean and maize seedlings and plants treated with low concentration of herbicide, either alone or supplemented with NaCl, were unaffected, whereas at high concentrations of the herbicide a significant decrease in chloroplast pigments was found. Nitrate reductase activity (NRA) was increased significantly at low concentrations of the herbicide alone and decreased significantly at high levels. Inclusion of NaCl into the herbicide media induced significant decreases in NRA of both castor bean and maize seedlings and plants. Unlike NRA changes, protease activity was increased significantly with high concentrations (5 and 10 g m^{-3}) of metribuzin and decreased significantly with its low (0.5 and 2.5 g m^{-3}) concentrations.

Introduction

The influence of metribuzin on the nitrogen metabolism varies according to the amount of herbicide applied, the amount and form of nitrogen supplied to the plant, growth conditions and the plant species (Ashton and Crafts 1973, Hamed 1990). Subtoxic levels of metribuzin increase growth and nitrogen content of certain species (Tweedy and Ries 1967, Klepper 1974, Moustafa *et al.* 1981, Hamed 1990).

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Apparently, this has resulted from an increase in NRA which occurred in plants grown on a nitrate rather than on ammonia nitrogen source. In pea plants, Lalova and Ekaterina (1984) found that the raised contents of total- and protein-N as well as other free amino acids, due to metribuzin application, were in correlation with the enhanced NRA activity.

The content of pigments is mostly reduced after herbicide treatments (Melin *et al.* 1981). It depends upon light-dependent reactions leading to the synthesis of chlorophyll (Chl) and formation of chloroplasts or their precursors (Virgin 1964).

Pallett and Dodge (1980) found a rapid decrease of Chl content in flax cotyledons following treatment with monuron; carotenoid (Car) breakdown preceded Chl loss. A noticeable absence of starch in chloroplasts of herbicide-treated cotyledone suggested the process of Chl biosynthesis was inhibited (Hoagland 1989); atrazine at 10^{-4} M decreased the level of Chl in soybean seedlings below that of the control after 96 h.

Little attention has been given to the physiological effects of herbicides on specific crops. Thus, the aim of this work was to investigate some metabolic responses of castor bean and maize plants to different rates of metribuzin herbicide, either alone or in combination with NaCl, supplemented into Hoagland nutrient media.

Materials and methods

Homogenous seeds of castor bean (*Ricinus communis* L. cv. Baladi) and maize (*Zea mays* L. cv. Giza 2) were sterilized and germinated on *Whatman No. 1* filter paper watered with 20 cm of Hoagland nutrient solution (1/4-strength) in plastic dishes. The germinating dishes of castor bean and maize were incubated in the dark at 25 °C for 4 d. Then 5 uniform germinating seeds were placed in a perspex plate suspended over a black-painted glass cylinder (600 cm^3) containing either Hoagland nutrient or Hoagland nutrient solution supplemented with herbicide and/or NaCl. Sampling of seedlings and plants was performed after 7 and 25 d from the date of sowing, as in Hasaneen *et al.* (1993).

Nitrogenous constituents were extracted as described by Yemm and Willis (1956). Total N and total soluble N were determined in the dry powdered tissues by the conventional micro-Kjeldahl method. Aliquots of the extracts were used for estimation of ammonia N by the method of Delory (1949), using the Nessler's reagent. Nitrate N was estimated by the phenol-disulphonic acid method as described by Snell and Snell (1949). Amino-N was measured by the method of Muting and Kaiser (1963). Subtracting total soluble N from total N gave the value for protein N.

Chl *a*, *b* and Car amounts were determined in leaves of the two test plants by the method of Metzner *et al.* (1965).

Extraction and assay of NRA in castor bean and maize plants were carried out by the method described by El-Shora (1981). Protease activity in castor bean and maize tissues was determined according to Gallop *et al.* (1957, using the Lowry assay (absorbance was measured at 700 nm).

The results were statistically analysed using the least significant difference (L.S.D.) at 5 and at 1 % levels (Snedecor and Cochrain 1980).

Results and discussion

Changes in nitrogen content: Treatment of castor bean, and maize seedlings and plants with low concentrations of metribuzin (0.5 and 2.5 g m^{-3}), either alone or supplemented with 50 g m^{-3} NaCl, induced significant decreases in NO_3^-, NH_4^-, amino- and total soluble-N contents, whereas high concentrations (5 and 10 g m^{-3}) of the herbicide in addition to NaCl induced significant increases in the above mentioned fractions (Table 1). On the other hand, protein and total N contents of castor bean, and maize seedlings and whole plants increased in response to treatment with low concentrations of the herbicide in absence or presence of NaCl, and in the meantime were significantly decreased with high concentrations (5 and 10 g m^{-3}) of metribuzin supplemented with NaCl, in relation to the control (Table 1).

In support of our results, Hiranpradit *et al.* (1972), Penner and Early (1972) and Terrillon and Payrot (1973) reported that total N and amino acid levels increased after the treatment of maize with atrazine (less than 3 g m^{-3}), maize and soybean with atrazine (10^{-6} - 10^{-4} M) and wheat with 4×10^{-7} M atrazine, respectively. Triazine herbicide induced an increase in protein synthesis and NRA in pea and sweet maize; these increments were accompanied by changes in the ultrastructure of parenchyma cells of the developing cotyledons (Wu *et al.* 1972).

The uptake and reduction of nitrate to amino groups and protein synthesis require energy which may be obtained from the breakdown of saccharides The decrease in the starch and soluble sugars contents in metribuzin-treated plants is consistent with a greater energy utilization for protein synthesis (Hasaneen *et al.* 1993).

Changes in NRA and protease: In castor bean, and maize seedlings and plants treated with herbicide alone at low concentrations (0.5 and 2.5 g m^{-3}) NRA significantly increased, whereas at high concentrations (5 and 10 g m^{-3}) of metribuzin a significant decrease was apparent (Table 2). Increase in NRA after application of triazine was reported by Ries *et al.* (1967) and after application of simazine by Tweedy and Ries (1967). Similar increase in NRA was observed as a result of glyphosate application at a low concentration to soybean (Hoagland 1989).

Inclusion of NaCl (50 g m^{-3}) into Hoagland media containing metribuzin at low and high concentrations led to progressive significant decreases in NRA of both castor bean, and maize seedlings and whole plants, as compared with the controls (Table 2).

The treatment of both castor bean, and maize seedlings and plants with metribuzin (0.5 and 2.5 g m^{-3}), either alone or in combination with NaCl, led to significant decreases in the protease activity, whereas high concentrations of the herbicide alone (5 and 10 g m^{-3}) or combined with NaCl induced significant increases in protease activity (Table 2).

The changes in enzymes activity by application of metribuzin alone and combined with NaCl may be attributed to the herbicide action on the biosynthesis of enzyme protein, enzyme activation and membrane permeability. Engelsma (1973) thought that the stimulation of tyrosine amonia lyase activity in excised segments of cucumber seedlings by dichobenil herbicide was to be regarded as a result of

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

Concentrations metribuzin [g m^{-3}]	NaCl [g m^{-3}]	Castor bean nitrate	ammonia	amino	total soluble	protein	total	Maize nitrate	ammonia	amino	total soluble	protein	total
Seedlings:													
0	0	444	288	152	3 365	10 502	13 867	345	511	289	4 592	9 423	14 015
0.5	0	323**	185**	85**	1 332**	14 455**	15 787**	234**	333**	174**	2 917**	13 252**	16 169**
2.5	0	393**	236*	127*	2 454**	11 971**	14 425*	282**	439**	233*	3 834**	10 351**	14 185*
5.0	0	554**	415**	180*	4 526**	7 300**	11 826**	449**	665**	352**	6 163**	6 443**	12 606**
10.0	0	729**	596**	255**	6 673**	3 137**	9 810**	663**	784**	475**	8 471**	2 512**	10 983**
L.S.D.													
at 5 % level		22	14	7	168	525	694	17	25	14	229	471	700
at 1 % level		33	21	11	252	787	1 041	25	38	21	344	706	1 050
0	50	658	536	213	5 724	4 834	10 558	588	753	412	8 099	3 370	11 469
0.5	50	495**	355**	164**	3 967**	8 833**	12 800**	394**	586**	305**	5 435**	7 792**	13 227**
2.5	50	604*	486*	189*	5 181**	6 005**	11 186**	526*	712*	361*	7 007**	5 185**	12 192**
5.0	50	771**	688**	271*	7 248**	2 190**	9 438**	718**	836**	524**	8 985*	1 711**	10 696**
10.0	50	823**	747**	316**	8 009**	1 185**	9 194**	771**	872**	598**	9 414**	853**	10 267**
L.S.D.													
at 5 % level		32	26	11	286	241	527	29	37	21	404	168	573
at 1 % level		49	40	16	429	362	791	43	56	30	606	252	869

Plants:													
0	0	1 623	643	265	5 148	15 621	20 769	1 003	1 243	513	7 744	13 854	21 598
0.5	0	1 201**	463**	182**	3 432**	20 431**	23 863**	765**	935**	386**	5 151**	17 942**	23 093**
2.5	0	1 435**	595*	243*	4 574**	17 344*	21 918*	894**	1 081**	454**	6 855**	15 634**	22 489**
5.0	0	1 948**	769**	338**	5 833*	11 724**	17 557**	1 202**	1 402**	611**	10 643**	9 207**	19 850**
10.0	0	2 532**	1 154**	431**	9 104**	6 085**	15 189**	1 379**	1 615**	882**	12 978**	4 550**	17 488**
L.S.D.													
at 5 % level		81	32	13	257	781	1 038	50	62	26	387	692	1 079
at 1 % level		121	48	19	385	1 171	1 557	75	93	38	581	1 038	1 619
0	50	2 335	981	404	8 001	7 946	15 947	1 311	1 537	743	12 385	5 691	18 076
0.5	50	1 709**	689**	285**	4 975**	13 473**	18 448**	1 094**	1 286**	569**	8 922**	11 676**	20 598**
2.5	50	2 183*	896*	353**	6 596**	10 203**	16 799*	1 146**	1 353**	605**	11 832**	7 132**	18 964**
5.0	50	2 685**	1 267**	492**	9 955**	4 453**	14 408*	1 455**	1 685**	919**	13 495**	3 272**	16 767**
10.0	50	2 771**	1 384**	561**	10 806**	2 872**	13 768**	1 607**	1 803**	1 033**	14 101**	2 181**	16 282**
L.S.D.													
at 5 % level		116	49	20	400	397	797	65	76	37	619	284	903
at 1 % level		174	73	30	601	595	1 195	98	114	55	928	424	1 355

Table 2. Unit activity determinations of nitrate reductase and protease 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 100 cm^3 enzyme preparation. * - $P = 0.5$, ** - $P = 0.1$.

Concentration in Hoagland solution		Nitrate reductase				Protease			
		Castor bean		Maize		Castor bean		Maize	
metribuzin	NaCl	seedlings	whole plants	seedlings	whole plants	seedlings	whole plants	seedlings	whole plants
0	0	406.4	537.0	347.3	477.3	122.3	180.8	95.8	159.8
0.5	0	597.9**	698.3**	481.8**	616.5**	75.5**	129.6**	43.1**	100.1**
2.5	0	530.1**	647.8**	433.6**	569.6**	100.9**	154.1**	60.2**	120.6**
5.0	0	300.0**	411.3**	262.5**	370.9**	176.8**	256.3**	150.2**	220.2**
10.0	0	224.3**	334.5**	194.4**	297.0**	270.8**	360.5**	240.1**	296.1**
L.S.D. at 5 % level		20.3	26.8	17.3	23.8	6.1	9.0	4.7	7.9
L.S.D. at 1 % level		30.4	40.2	25.9	35.7	9.0	13.5	7.1	11.9
0	50	455.1	587.1	389.7	500.4	265.0	354.2	213.6	286.6
0.5	50	354.0**	469.1**	309.1**	416.2**	141.0**	203.9**	124.5**	183.1**
2.5	50	269.2**	385.5**	231.8**	331.6**	223.7**	300.1**	193.9**	210.7**
5.0	50	197.3**	273.3**	135.4**	245.9**	334.1**	460.2**	281.2**	320.3**
10.0	50	145.5**	225.7**	100.6**	194.7**	388.6**	492.3**	301.6**	390.6**
L.S.D. at 5 % level		22.7	29.3	19.4	25.0	13.2	17.7	10.6	14.3
L.S.D. at 1 % level		34.1	44.0	29.1	37.5	18.8	26.6	16.0	21.4

increasing membrane permeability. The inhibitory effect of herbicide treatments on synthesis of proteins necessary for enzymes and co-enzymes could be regarded as a possible explanation for reductions in the activity of several enzymes. As a result of herbicide treatment the activity of several enzyme systems was modified: *e.g.* ATPase in bush bean (Singh and Salunkhe 1970), and protease in germinating wheat and mung bean (Dalvi *et al.* 1972).

Table 3. Effects of different concentrations of metribuzin, either alone or in combination with NaCl, on pigment contents (Chl - chlorophyll, Car - carotenoid) [mg kg^{-1}(fr.m.)] of castor bean and maize seedlings (7 d) and plants (25 d). * - $P = 0.5$, ** - $P = 0.1$.

Concentrations [g m^{-3}] in Hoagland solution of metribuzin	NaCl	Castor bean Chl *a*	Chl *b*	Car	Maize Chl *a*	Chl *b*	Car
Seedlings							
0	0	751	316	217	881	386	281
0.5	0	792	321	218	902	391	280
2.5	0	801	331	220	943	402	279
5.0	0	532**	297**	126**	631**	351**	192**
10.0	0	410**	262**	98**	492**	302**	103**
L.S.D. at 5 % level		37	15	11	44	19	14
L.S.D. at 1 % level		56	23	16	65	28	21
0	50	612	209	299	701	262	341
0.5	50	631	212	301	723	271	350
2.5	50	640	221	313	752	283	362
5.0	50	401**	184**	228**	501**	221**	281**
10.0	50	306**	126**	126**	417**	198**	207**
L.S.D. at 5 % level		31	10	14	35	13	17
L.S.D. at 1 % level		45	15	22	52	19	25
Plants							
0	0	963	447	319	1002	531	402
0.5	0	972	443	309	1031	552	409
2.5	0	983	416	310	1052	563	413
5.0	0	802**	272**	241**	872**	413**	313**
10.0	0	733**	312**	204**	713**	328**	255**
L.S.D. at 5 % level		48	22	16	50	26	20
L.S.D. at 1 % level		72	33	24	75	39	30
0.	50	817	380	372	892	472	466
0.5	50	831	382	381	912	481	471
2.5	50	849	389	376	925	499	479
5.0	50	721**	301**	302**	713**	381**	331**
10.0	50	602**	217**	261**	609**	303**	243**
L.S.D. at 5 % level		40	19	18	45	24	23
L.S.D. at 1 % level		61	28	27	66	35	34

Changes in pigment contents: Low concentratiuons of metribuzin, either alone or with NaCl induced non-significant changes in Chl *a*, *b* and Car in leaves of both plants and seedlings of castor bean and maize (Table 3). High concentrations of metribuzin (5 and 10 g m^{-3}), either singly or fortified with NaCl, induced significant decreases in chloroplast pigment contents. NaCl-salinity induced more reductions in chloroplast pigments of both plant species.

In this connection, Nemat-Allah (1991) found out that the Chl ($a + b$) and Car contents of the herbicide-treated maize and soybean seedlings were markedly reduced. This has been confirmed by our results with castor bean and maize.

In conclusion, it seems that the effects of herbicide and/or NaCl on castor bean and maize seedlings and plants are dependent on the concentration of herbicide and/or NaCl used, on the plant age and on the duration of treatment.

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