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EFFECT OF CR(III) COMBINE WITH ATRAZINE ON PROTEIN, CARBOHYDRATE, AMINO ACID AND CHLOROPHYLL CONTENTS IN *VIGNA RADITA* (L.) WILCZEK

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ABSTRACT

The effect of Cr (III) combined with atrazine on the level of carbohydrates, proteins, amino acids in roots and shoots, and that of chlorophyll, of bean plants were investigated. Application of Cr $(NO_3)_3$ amounting to 10 to 200 ppm, combined with atrazine at 10 to 100 ppm, resulted into a decrease in carbohydrate, protein and amino acid contents in shoots and enhancement in roots. Chlorophyll "a" content decreased and chlorophyll "b" was increased in bean plants with the exclusive presence of chromium and that chromium combined with atrazine lowered both of these. A significant decrease ($p<0.01$) in length of shoot and an increase in root length was observed with an increase concentration of Cr (III) and atrazine. A little growth stimulant effect was also observed at 5ppm concentration of atrazine.

Key words: Atrazine, chromium, carbohydrates, amino acids, protein, chlorophyll, *Vigna radita.*

INTRODUCTION

The major causes of elevated concentration of heavy metals in soil include soil property, agricultural activities, manufacturing of industrial products and the use of sewage sludge as fertilizer in agricultural field (Shaukat *et al.*,1999). Chromium is introduced to the soil through its use in chrome plating, steel, leather tanning, film and photographic equipment and metal cleaning. Chromium being one of the heavy metals is likely to cause severe toxic effects on plants such as reduction in plant growth and seed germination (Iqbal *et al.*,1991; Iqbal and Siddiqui, 1992; Shaukat *et al.*, 1999).

The reduction in plant growth may be attributed to alteration of metabolic pathway including respiration and photosynthesis by disturbing the cellular enzymes (Tomasevic *et al.*, 1991; Kurpa *et al.*, 1993). Atrazine is a chloro-amino triazine herbicide, is primarily a soil applied herbicide. It is a selective and pre emergence herbicide for control of many grasses and broad leafed weeds in maize, sorghum, sugar cane and many crops and increases the yield of crops (Shah *et al*., 2000). It is apoplectically translocated and leaching of atrazine is limited. It concentrates firstly in the internal veinal areas and finally in the margin of the leaf. There is no apparent simplistic movement out of the leaf (Ross and Lambi, 1985). Atrazine is a photosynthetic inhibitor and causes chlorosis and desiccation of green tissues. However all these effects are observed in light and not in dark. Atrazine causes degradation of chloroplast and also produces swelling followed by disruption of general disk.

Mc Closky *et al.* (1990) found that atrazine concentration amounting to more than 0.05μM inhibits protoplast O_2 evolution because of its binding to photo system II reaction. Its specific site of action is oxygen evolution or photolysis in hill reaction during photosynthesis. It also inhibits ATP formation. Giannopolitis and Ayer (1978) reported atrazine-based inhibition of photosynthesis electron transport and increase in photo-oxidation in chloroplast. Atrazine also has growth stimulant effect at low concentration in the form of increased length shoot and root leaf blade and stem diameter. However higher concentrations result in inhibition of metabolic processes. The greening effect in atrazine treated plants is either due to increased uptake of nitrogen by the root or delay in catabolism of chlorophyll and senescence of plant. Einhelling (1989) and Raveton *et al. (*1997) studied the transformation and detoxification of atrazine in seedling. Since chromium is the main effluent of many industries and atrazine is commonly used as herbicide for the crop plants , the objectives of these studies were:

(i) To determine the adverse effects of atrazine combine with chromium on growth of bean plants,
(ii) To determine its effects on carbohydrates, amino acids, proteins and chlorophyll contents of bean plants.

MATERIALS AND METHODS

Pot experiments were conducted in growth chamber (30°C day and 25°C night) for one week in 2003. Five different concentrations of atrazine solutions (80%wp) 0, 5, 10 and 100 ppm were prepared in half strength hoagland solution wich already contained 0,5, 10,50, 100, 150, 200ppm chromium (Chapman, 1976) Few drops of tween-20 was used as dispersing agent. Ten to fifteen seeds of bean seeds soaked in water for 4h were surface sterilized with 0.3 % calcium hypochlorite for 5 min. and rinsed with distilled water. They were distributed into different pots containing 0.5, 10, 50 and 100 ppm atrazine solutions. Plants were analyzed after one week. The anthrone reactions (Tandon, 1993) are the basis of the rapid and convenient method for the determination of hexoses, aldopentoses and hexauronic acids either free or present in polysaccharides.

Table 1. Effect of atrazine and chromium on root and shoot length of bean Plants.

Concentration of chromium	Concentration of atrazine	Average length of shoot (cm)	Average root length (cm)
5 ppm	0	26.8 ± 1.01	3.9 ± 1.31
	5	27.02 ± 0.80	4.38 ± 1.24
	10	25.04 ± 0.51	4.66 ± 1.40
	50	15.78 ± 0.78	5.3 ± 1.51
	100	6.24 ± 1.02	5.54 ± 1.23
10 ppm	0	21.48 ± 1.40	4.36 ± 1.32
	5	20.5 ± 1.81	4.78 ± 1.23
	10	20.4 ± 2.03	4.9 ± 1.25
	50	12.3 ± 1.51	5.46 ± 1.11
	100	6.16 ± 1.73	5.62 ± 1.23
50 ppm	0	19.06 ± 2.10	4.5 ± 1.20
	5	18.8 ± 1 .45	4.84 ± 0.98
	10	16.54 ± 1.22	5.3 ± 1.21
	50	11.04 ± 1.24	5.48 ± 1.21
	100	5.1 ± 0.56	7.16 ± 1.34
100 ppm	0	17.4 ± 1.24	5.78 ± 1.21
	5	17.3 ± 1.35	6.24 ± 1.44
	10	15.1 ± 1.45	7.38 ± 1.23
	50	7.74 ± 1.25	7.7 ± 1.32
	100	3.42 ± 1.43	7.84 ± 1.41
150 ppm	0	16.4 ± 1.42	6.12 ± 1.23
	5	16.2 ± 1.34	7.31 ± 1.45
	10	14.3 ± 1.42	8.12 ± 1.23
	50	6.3 ± 1.25	8.9 ± 1.46
	100	3.2 ± 1.23	9.2 ± 1.23
200 ppm	0	15.8 ± 1.45	6.78 ± 1.32
	5	17.3 ± 1.45	7.24 ± 1.23
	10	13.43 ± 2.5	7.38 ± 1.45
	50	5.3 ± 1.33	8.2 ± 1.23
	100	2.2 ± 0.42	8.84 ± 1.23

Table 2. Effect of a chromium on Carbohydrates, amino acids and protein contents in shoots and root of bean plants.

In plant's	Concentration of chromium	Percentage of carbohydrates	Percentage of proteins	Percentage of amino acids
Shoots	0	0.468	1.3	8.8
	5	0.465	1.07	8.18
	10	0.380	0.92	5.82
	50	0.312	0.79	4.92
	100	0.22	0.803	3.10
	150	0.15	0.71	1.4
	200	0.08	0.64	0.97
Roots	0	0.21	1.78	
	5	0.41	1.62	5.09
	10	0.45	1.7	5.32
	50	0.48	1.87	6.5
	100	0.51	2.1	7.2
	150	0.59	2.23	7.54
	200	0.62	2.45	7.84

For the determination of chlorophyll an extract of plant in acetone is just required. Absorbance of the plant extract was recorded spectrophotometrically at two wave lengths of 663 and 654nm and the amount of chlorophyll is determined mg/gm by weight by using the standard formula (Bohlar and Elisabeth, 19870).

For proteins roots/ shoot extract in water was treated with Folin cicalteau Phenol reagent (half diluted). The extract was left for 30 min. at room temperature. A blue coloured complex was developed. Absorbance of the complex was observed at 650nm on Schimadzu 160 A UV-Visible spectrophotometer.

Carbohydrates were analyzed in protein free filtrate extract of plants in water using anothrone reagent. The complex that is formed can be estimated spectrophotometrically at 620nm (Tandon, 1993). For amino acid determination, water extract of root or shoot was treated with ninhydrin solution in 10% ethanol, and warmed at 50 to 70°C for few minutes till purple color appeared. Optical density was measured at 566 nm (Chapman, 1976).

RESULTS AND DISCUSSION

Effect of chromium:

Chromium at 5 ppm level produced no effect on plants (Table 1). At higher concentrations (50 – 200 ppm) it inhibited the shoot length whereas root length got increased. Carbohydrates amino acids and protein content were found to decrease in shoot and could have caused the decrease in shoot length. This inhibition effect is enhanced in the presence of chromium. The inhibitory effect of salt on seed germination could be the result of ionic (Redman, 1994) or osmotic effect (Micheal *et al.*, 1972), or it may be due to the decreased levels of auxine which get reduced by metal ions (Mukerji and Das Gupta, 1972). Carbohydrate levels decreased in shoots and increased in roots of bean plant. The increase in these contents in roots may attributed to increase in root length of bean plant (Tables 1-2).

Table 3. Effect of chromium combined with atrazine on carbohydrate, amino acids and protein content in roots of bean plants.

Concentration of atrazine (ppm)	Concentration of chromium	%age of Protein	% mg of amino acids	%age of carbohydrates
0	0	1.45	5.09	0.426
	5	1.62	5.32	0.43
	10	0.941	6.32	0.46
	50	0.75	6.35	0.49
	100	0.85	7.2	0.52
5	0	1.56	6.01	0.433
	5	1.83	6.2	0.395
	10	1.27	7.25	0.452
	50	1.62	8.5	0.47
	100	2.31	9.2	0.485
10	0	1.44	10.87	0.329
	5	2.07	12.3	0.385
	10	1.46	12.5	0.432
	50	2.13	15.2	0.0.445
	100	2.39	13.3	0.467
50	0	1.12	11.57	0.22
	5	2.33	14.5	0.378
	10	1.575	13.4	0.412
	50	2.42	14.2	0.432
	100	2.56	15.2	0.49
100	0	0.87	16.24	0.14
	5	2.11	16.5	0.375
	10	2.46	17.4	0.39
	50	2.50.	18.5	0.422
	100	2.67	19.2	0.438

Effect of Chromium combine with atrazine:

Chromium at 5 ppm level produced no effect on the plant (Tables 1-4). At higher concentrations (50 – 200 ppm) it inhibits the shoot length with the enhanced concentration of atrazine. But the root length gets increased as compared to the case when only atrazine is used (Table 1). Increase in root may appear because of increase in the amino acid, protein, carbohydrate contents in root (Table 3-4). This effect is more pronounced in presence of atrazine in bean plant in both roots and shoots (Tables 3-4). Although protein, carbohydrates contents have been reported to decrease in presence of atrazine with out chromium. Atrazine at higher concentration inhibited the seedling growth (Perveen *et al.,* 2002). This inhibition effect is enhanced in the presence of chromium. The inhibitory effect of salt on seed germination could be the result of ionic (Redman, 1994) or due to an osmotic effect (Micheal *et al.*,1972) or it may be due to decreased levels of auxine resulting by destruction of auxine by metal ions

(Mukerji and Das Gupta, 1972). Carbohydrates level decreased in shoots and increased in roots of bean plant with enhanced concentration of atrazine (10 – 100) and chromium (10 - 200 ppm) (Table 4). Carbohydrates are synthesized during the process of photosynthesis, both atrazine and chromium are the inhibitors of the photosynthesis by the degradation of chloroplast. This decrease in carbohydrate levels may be due to the disturbance in the enzyme system (Tomasevic *et al.*1991; Kurpa *et al.*, 1993). The degradation of chloroplast is manifested by decrease in chlorophyll a and b contents in plants at higher concentrations of atrzine (10 – 100 pm) and chromium (10- 200 ppm) (Table 5). The enhanced levels of carbohydrate in roots may be due to the basipital transportation of carbohydrates. Similarly proteins and amino acid contents are decreased in shoots and the increase in roots at higher concentrations of atrazine (10 – 100 ppm) and chromium (10 – 200 ppm) (Tables 3-4). It also may be due to the disturbance in enzyme system, because the atrazine inhibits the nitrite reductase activity (Kelpper, 1975), which is involved in amino acids production, and this effect gets enhanced in presence of chromium (Table 3-4). The increase in amino acids, proteins content in roots of bean plant may be due to the basipital translocation (Shaukat *et al.*, 1975). Atrazine reduces the growth, carbohydrates, amino acids and proteins contents of shoot of bean plant. This effect is more pronounced in the presence of chromium, not only length in roots increases but carbohydrates, amino acids and protein contents are increased as compared to when only atrazine was added. The combination of atrazine and chromium seems to be highly toxic to the bean plant.

Table 4. Effect of chromium combined with atrazine on carbohydrate, protein and amino acid content in shoots of bean plants.

Concentration of atrazine (ppm)	Concentration of chromium	%age of Protein	%age of amino acids	%age ofcarbohydrates
0	0	1.3	8.8	0.468
	5	1.07	8.18	0.38
	10	0.89	5.82	030
	50	0.70	4.92	0.27
	100	0.52	3.1	0.19
5	0	1.1	8.18	0.465
	5	0.92	4.52	0.36
	10	0.74	4.35	0.30
	50	0.63	4.07	0.26
	100	0.50	3.04	0.17
10	0	0.89	5.822	0.30
	5	0.71	4.35	0.26
	10	0.52	4.12	0.18
	50	0.32	3.7	0.16
	100	0.29	2.84	0.14
50	0	0.72	4.92	0.30
	5	0.52	3.82	0.2
	10	0.45	3.89	0.18
	50	0.29	3.14	0.165
	100	0.22	2.55	0.108
100	0	0.53	3.1	0.22
	5	0.45	3.24	0.14
	10	0.30	3.07	0.163
	50	0.19	3.75	0.13
	100	0.07	2.14	0.08

Table 5. Effect of chromium combined with atrazine on chlorophyll content of bean plants.

Concentration of atrazine (ppm)	Concentration of chromium	%age of chlorophyll "b"	%age of chlorophyll "a"
0	0	0.22	0.53
	5	0.131	0.522
	10	0.18	0.337
	50	0.197	0.204
	100	0.11	0.303
	150	0.08	0.28
	200	0.055	0.19
5	0	0.11	0.27
	5	0.122	0.516
	10	0.015	0.118
	50	0.11	0.20
	100	0.09	0.296
10	0	0.09	0.239
	5	0.105	0.363
	10	0.012	0.09
	50	0.087	0.145
	100	0.098	0.286
50	0	0.087	0.21
	5	0.04	0.147
	10	0.11	0.151
	50	0.07	0.183
	100	0.098	0.283
100	0	0.066	0.156
	5	0.027	0.11
	10	0.01	0.18
	50	0.008	0.17
	100	0.007	0.14

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