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Effect of fluoride toxicity on chlorophyll, protein percentage and energy content of Wheat (*Triticum aestivum* L.) and Chick pea (*Cicer arietinum* L.)

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Fluoride and SO_2 are air pollutants which are toxic to vegetation human lives and animals. 100-200 ppm doses of NaF are more toxic than 10 ppm. 10 ppm dose does not affect any plant or animal. Thus it is considered under threshold limit. The present experiment was conducted at C.C.R. (P.G.) College, Muzaffarnagar during the years 2002-2003 to study the effect of fluoride toxicity on chlorophyll, protein percentage and energy content in wheat and Chickpea. The Chlorophyll content in green leaves was studied on 60th day of sowing. Protein and energy contents were studied after harvesting with the oven dried plant material at 80 ^{0}C temperature 100-200 ppm concentrations of NaF were found toxic to wheat and chickpea.

Key words : Chlorophyll, Protein, Energy content, Fluoride toxicity.

INTRODUCTION

Fluoride kills in acute poisoning by blocking normal metabolism of cells. Enzymes involved in essential processes are inhibited. Vital functions such as the origin of transmission of nerve impulses cease (WHO, 1970). Toxicity health hazards were also noted (Spomer, 1973).

Wallac and Romney (1980) observed the chlorosis due to fluoride toxicity in various plants. They observed the inward rolling, discoloration of leaves prior to death of leaf in rice and wheat. The initiation of symptoms of toxicity begin from tips and margins of leaves was found in gladiolus (Hitchcock *et al.*, 1963). Necrosis in cereal crops like wheat, flacking or chlorotic mottling in corn leaf tips, reddish brown bands in *Simalacina* sp are characteristic features of mild fluoride toxicity. In case of moderate toxicity chlorotic spots develop between the veins. When injury is severe some of chlorotic tissues become necrotic, particularly along the margins and leaf tips.

Treshow and Pack (1968) discussed the symptoms of injury on vegetation of gaseous fluoride. Fluoride air pollutants enter the plant primarily though the leaves. It enters through the stomata, passes into the intercellular spaces and is absorbed by the mesophyll cells (Thomas and Hendricks, 1956). From the mesophyll the fluoride may move to other cells by simple diffusion or through the vascular tissue. It moves with the transpiration stream towards the leaf tips and margins where it accumulates in concentrations at least several times higher than the average concentration in the leaf as a whole (Zimmerman and Hitchcock, 1956). Injury from fluorides appears on leaf tips or margins of sensitive plants, since toxic ions migrate to those regions on the leaf. Gladiolus varieties are very sensitive to fluoride at a concentration of several parts in a billion parts of air (Daines *et al.*, 1960). The effects of atmospheric pollution on vegetation were studied by Leone (1980) and he suggested that fluoride may accumulate in high concentrations in plant parts.

MATERIALS AND METHODS

The seeds of wheat c.v. WL75, UP2003 and Chickpea c.v. 256, K 850 were obtained from I.A.R.I. New Delhi. The experiments were sown in Randomizded Block Design with four replications at C.C.R. (P.G.) College, Muzaffarnagar during the year 2002-2003. Six concentrations of NaF along with control were taken. The solutions of sodium fluoride were sprayed fortnightly after one month of sowing the crops. The concentrations of NaF were C, 10, 25, 50, 100 and 250 ppm. The methods adopted for the estimation of chlorophyll, nitrogen content, protein percentage and energy content are given below:

Estimation of total chlorophyll:

The chlorophyll content in fresh leaves was determined according to Arnon (1949) on the absorption of light by aqueous acetone (80%) extracts of chlorophyll. Organic solvent 4:1 Acetone and alcohol was used.

0.5 g fresh leaves of control and treated plants were

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taken with organic solvent in clean specimen tubes. The extracts were centrifuged at 3000 rpm for 15 minutes and each volume was made upto 25 ml of each sample by adding more organic solvent.

Carlzeiss PMQ 2 spectrophotometer was used at Institute of Life Science, J.N.U. New Delhi and the observations of total chlorophyll content were recorded on 645, 652 and 663 wave lengths, respectively.

Total chorophyll content was calculated by using the following formula (Arnon, 1949).

C = 20.2 D 645 + 8.02 D 663 in mg/g dry weight

Estimation of total nitrogen and protein:

The estimation of total nitrogen of plant material is done in three steps (i) Digestion (ii) Distillation and (iii) Titration. Nitrogen percentage and the amount of protein content synthesized by the plant tissues were determined according to Jackson (1958) and Misra (1968).

Digestion:

500 mg dried and powdered plant material was taken in 50 ml Kjeldahl flasks with 5 ml of H_2SO_4. O.1 g catalyst mixture of copper sulphate, potash sulphate and selenium dioxide in the ratio of 1:8:1, respectively was also added.

Distillation:

After complete digestion of the plant material the volumes were made upto 50 ml. The Distillation was done in a Markham apparatus as described by Jackson (1958) and Misra (1968).

RESULTS AND DISCUSSION

Titration:-

In the process of distillation, the vapours of digested plant material were collected in boric acid solution in 100 ml beaker. Titration was done with N/10 HCl nitrogen percentage calculated:

Nitrogen percentage = (T-B) x 5 x N x 1.4/S

where,

T = Volume of HCl (Standard Acid used in actual titration)

B = Blank

N = Normality of NaOH = -N/10

1.4= Constant (Atomic weight of N_2)

S = Dry weight of plant sample in g.

The difference (T-B) was multiplied by 5 because only 10 ml digested material out of 50 ml was distilled.

The protein content was determined by multiplying total nitrogen by 6.25.

Determination of caloric value:

The caloric values were determined by Bomb Calorimeter.

The samples were oven dried for 48 hours at 105°C and then reduced to powder by an electric grinder. After passing through 40 mesh, these samples were stored in stoppered bottles subjected to caloric analysis. The plant material from each sample was compressed into pellets about 1 gm of weight and dried in an oven at 105°C for 24 hours. Afterwards the pellets were kept in a desiccator. The caloric values were determined by igniting the pallets of the plant material in an oxygen Bomb Calorimeter. Caloric value per gm dry weight of plant material is given:

$$\text{Energy or } C = \frac{W_1 (T_2-T_1) + W_2 (T_2-T_1)}{W}$$

where,

W = Weight of pellet

W_1= Water equivalent (531.28)

W_2 = Weight of water (1200 ml)

T_2-T_1 = Temperature difference.

The effect of NaF on Chlorophyll content, Protein percentage % and energy content in Wheat and Chickpea has been presented in Table 1. The Chlorophyll content

Table 1 : Mean chlorophyll content (mg/g dry weight), protein % and energy content in Cal/g of dry weight

Treatments (NaF)	Chlorophyll				Protein %				Energy content			
	Wheat		Chickpea		Wheat		Chickpea		Wheat		Chickpea	
	WL 75	UP 2003	Pusa 256	K 850	WL 75	UP 2003	Pusa 256	K 850	WL 75	UP 2003	Pusa 256	K 850
Control	7.26	7.27	7.98	6.98	11.75	11.75	13.37	13.56	3392.0	3395.0	3385.5	3380.2
10 ppm	7.24	7.26	6.77	6.77	11.75	11.68	11.00	10.32	3168.0	3180.0	3113.6	3115.6
25 ppm	7.20	7.22	6.75	6.75	11.50	11.50	7.75	8.25	3075.0	3068.0	3007.8	3015.0
50 ppm	7.16	7.21	6.58	6.57	10.00	10.06	7.62	7.62	2980.0	2979.0	2937.5	2936.2
100 ppm	7.16	7.20	6.51	6.50	7.50	7.87	7.50	7.31	2706.0	2698.0	2683.0	2682.2
200 ppm	7.15	7.17	6.28	6.30	7.06	7.12	7.06	7.06	2318.0	2322.0	2305.5	2301.4

was recorded at the age of 60 days after sowing of the seeds in the experimental field. The Chlorophyll content was found maximum 7.27 in control treatment and minimum 7.17 in 200 ppm in Wheat variety UP 2003. Similar results were found in both varieties of Chickpea.

The effect of NaF on Protein % was also found maximum (11.75) in control and 7.06 in WL75. Similarly protein content 13.56 was found in control in Chickpea variety K 850. Minimum protein content was found 7.06 in 200 ppm NaF dose.

Maximum energy content was found in control (3395.0) in wheat variety UP 2003 and minimum (2318.2) in 200 ppm in WL75. Similarly maximum (3385.0) in control treatment and minimum (2301.4) in 200 ppm in Chickpea variety K 850 was recorded.

It has been seen in the present trial that Chlorosis had affected the Wheat and Chickpea crops both in 100-200 ppm concentrations of NaF. Nacrotic lesions were also seen on the leaf lamena. Due to severe attack of fluoride, the burning of leaf tips and margins was very common in 200 ppm concentration. Similar effects were already reported by Arya (1971, 1997), Singh (1992) and Malik (1997).

Other research works of similar nature are as that of Nimesh (2001) on pea (*Pisum saturum* L.) and barley (*Hardeum vulgare* L.), Chaudhary (2002) on sugar beet and garlic, Saini (2003) on onion (*Allium cepa* L.); Chaudhary (2004) on wheat and chickpea, Tyagi (2004) on urdbean (*Vigna mungo* L.), Saini and Singh (2005) on onion (*Allium cepa* L.) and Singh (2006) on cluster bean and lentil.

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