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EFFECT OF HERBICIDE GOAL (OXYFLUORFEN) ON DNA, RNA AND PROTEIN CONTENTS OF SEEDLINGS OF *Hibiscus canabinus* Linn.

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ABSTRACT

Effect of oxyfluorfen on macromolecular (DNA, RNA and Protein) contents of treated seedling was studied. As the concentration increase the percentage of DNA, RNA and Protein gradually decrease. The percentage of DNA, RNA and Protein content of control seedling was 9.9903 x 10^{-3}, 8.4054 x 10^{-3} and 4.34 respectively.

In oxyfluorfen the percentage of DNA decrease from 5.9263 x 10^{-3} to 4.9886 x 10^{-3} at 1000 to 8000 ppm, respectively. The percentage of RNA was decrease from 7.4577 x 10^{-3} to 6.6381 x 10^{-3} at 1000 to 8000 ppm, respectively. The decrease in protein contents of seedling was from 3.51to2.97 at 1000 to 8000 ppm of oxyfluorfen, respectively.

Key words: Herbicide, DNA , RNA , Protein , *Hibiscus cannabinus* Linn.

INTRODUCTION

The seeds of *Hibiscus cannabinus* Linn. treated with different concentrations of oxyfluorfen (1000 to 8000 ppm) for 24 hours. After treatment seeds were washed thoroughly with distilled water and kept for the germination in petridishes lined with double layers of moistened filter paper under laboratory condition. Seeds soaded in distilled water used as control. The treated and untreated seeds were allow to grow for seven days.

Each sample containing one gram fresh weight of seven days old seedling were taken for extraction, and estimation of nucleic acids and total proteins. The number of seedling per gram were counted and noted every time. For extraction of nucleic acids, the method suggested by Ogur and Rosen (1950) and for the protein extraction, the Kjeldalh's method adopted by Schneider (1945) were followed. The three replicates were used for each sample at each concentration of herbicide.

Extraction and estimation of nucleic acids

The weighted samples were frist homogenized in 15ml of 10% perchloric acid (PCA) at 0°C in glass pestal and mortal and centrifuged the homogenate at 2°C to 4°C for 15 minutes. The extracts were discarded and resuspended. The residue on cold 5% PCA and centrifuged again for 10 to 15 minutes. The supernatent was discarded and residue was washed sequentially with 70%ethanol , 95%ethanol and finally with boiling ethanol-ether (3:1) in water bath twice and then with cold 0.2N PCA. The residue was suspended with cold 2N PCA and stored at 2-5°C for 18 hours. After it the solution was centrifuged and supernatent was collected. The residue was resuspended with cold 2N PCA and centrifuged and two supernatents were combined and made volume up to 20 ml with distilled water. This supernatent containing RNA fraction was used for quantitative estimation of total RNA. The residue was suspended with 1N PCA and heated at 70°C for 20 minutes and the solution was centrifuged. The supernatent was collected

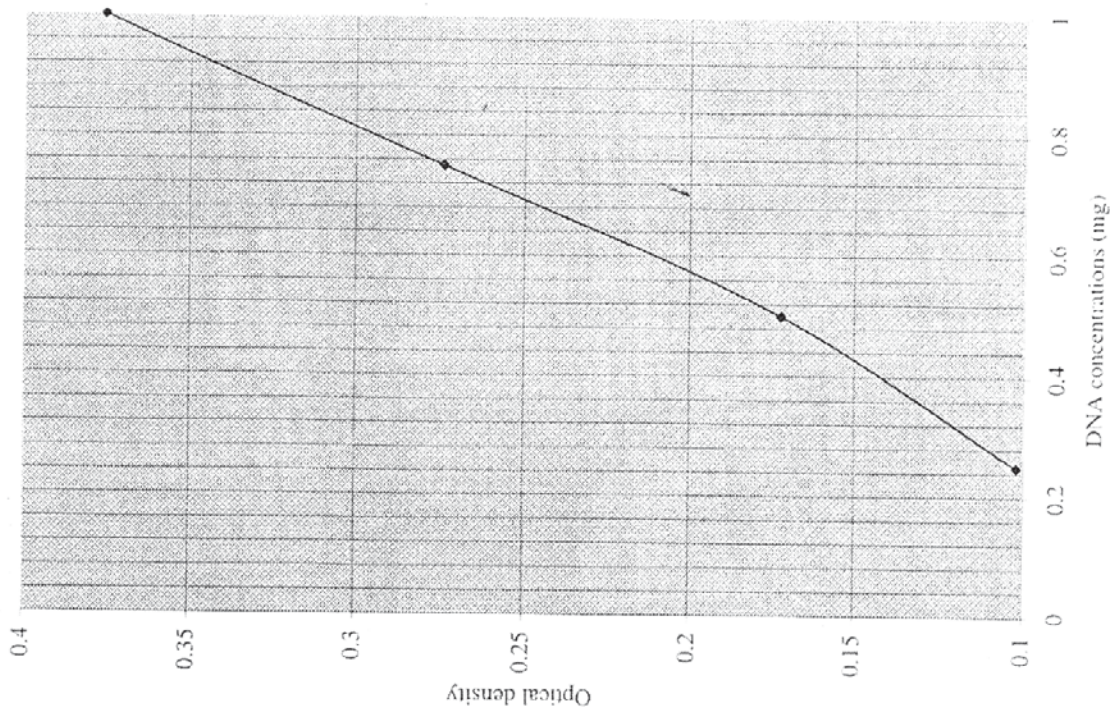

Fig. - 1: Standard graph (Calf thymus DNA)

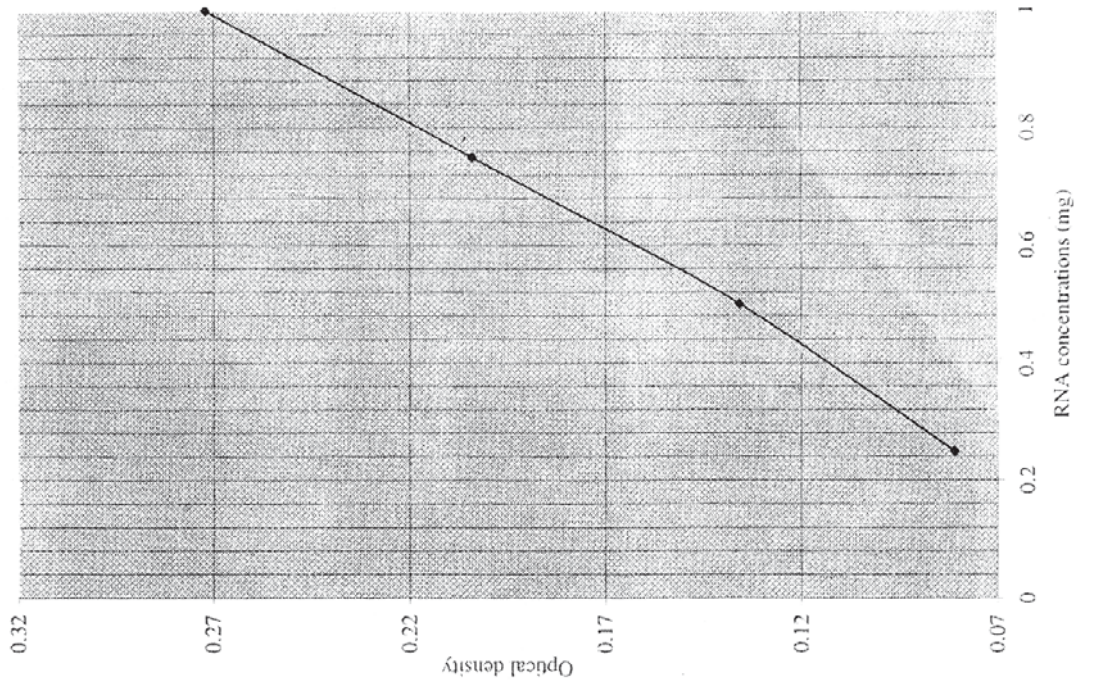

Fig. - 2: Standard graph (yeast RNA)

Table - 1: Showing DNA percentage in the seedlings of *Hibiscus cannabinus* due to application of herbicide.

Herbicide	Concentrations (ppm)	% of DNA per seedling	Standard error (±)
	Control	9.9903×10^{-3}	2.1312×10^{-3}
Oxyfluorfen	1000	5.9263×10^{-3}	2.569×10^{-3}
(Goal)	2000	5.6009×10^{-3}	2.4494×10^{-3}
	4000	5.2904×10^{-3}	2.2803×10^{-3}
	6000	5.1241×10^{-3}	2.8106×10^{-3}
	8000	4.9865×10^{-3}	2.9694×10^{-3}

Table - 2: Showing RNA percentage in the seedlings of *Hibiscus cannabinus* due to application of oxyfluorfen

Herbicide	Concentrations (ppm)	% of RNA per seedling	Standard error (±)
	Control	8.4054×10^{-3}	1.4232×10^{-3}
Oxyfluorfen	1000	7.4577×10^{-3}	1.3191×10^{-3}
(Goal)	2000	7.3635×10^{-3}	2.8809×10^{-3}
	4000	7.3393×10^{-3}	5.6833×10^{-3}
	6000	6.9302×10^{-3}	2.8809×10^{-3}
	8000	6.6381×10^{-3}	2.3664×10^{-3}

Table - 3: Showing percentage of protein per seedlings in *Hibiscus cannabinus* following herbicide treatment

Herbicide	Concentrations (ppm)	% of protein per seedling	Standard error (±)
	Control	4.34	0.03
Oxyfluorfen	1000	3.51	0.0
(Goal)	2000	3.39	0.05
	4000	3.34	0.05
	6000	3.16	0.01
	8000	2.97	0.05

and residue was resuspended with 1N PCA and centrifuged. Both supernatent was combined and make volume 20 ml by adding distilled water which was comprised DNA fraction and it was used for extraction of total DNA. All centrifugens were carried out at 0°C at 20,000 RPM with 80% speed by refrigerated centrifuge model.

The total RNA and DNA in PCA extract were estimated by measuring absorption at 600 nm and 620 nm, respectively with the help of "ELICO UV" spectrophotometer. The DNA and RNA content of the samples were calculated from standard graph of calf-thymus DNA and yeast RNA and is represented graphically.

DNA

Samples of PCA extract containing DNA were taken for dilution which was ranging from 0.25, 0.50, 0.75 and 1.0 ml taken in different test tubes and volume was made upto 2 ml with distilled water. In blank was prepared by using 2 ml distilled water. In each test tube added the 2 ml of diphenylamine reagent. The samples were kept at room temperature for 16 hours in dark and determined absorption at 620 nm. The DNA content of samples

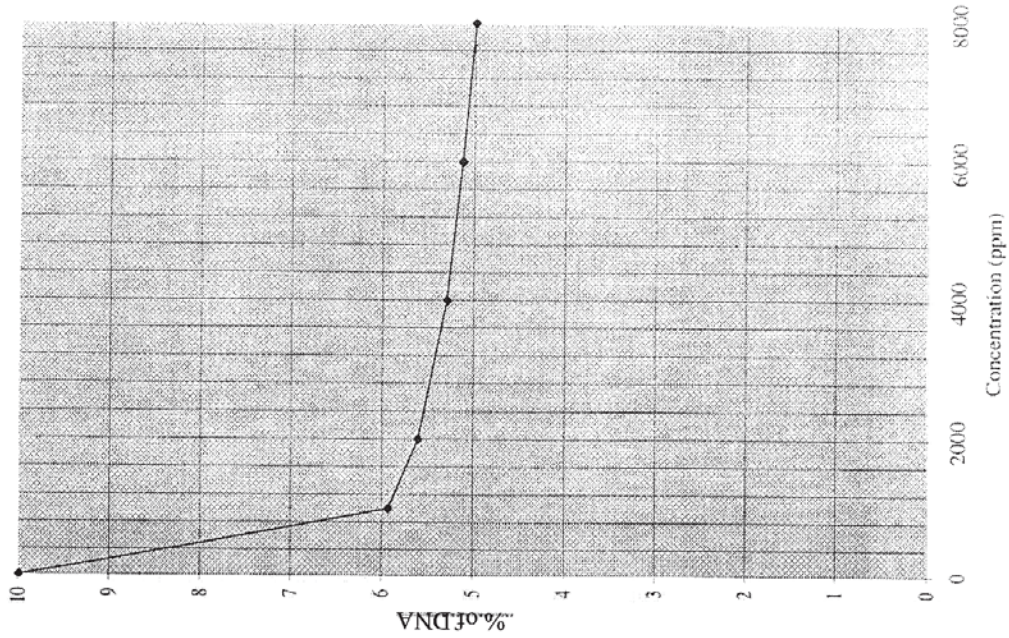

Fig. - 3: Graph showing DNA percentage per seedling at different concentrations of goal (oxyfluorfen)

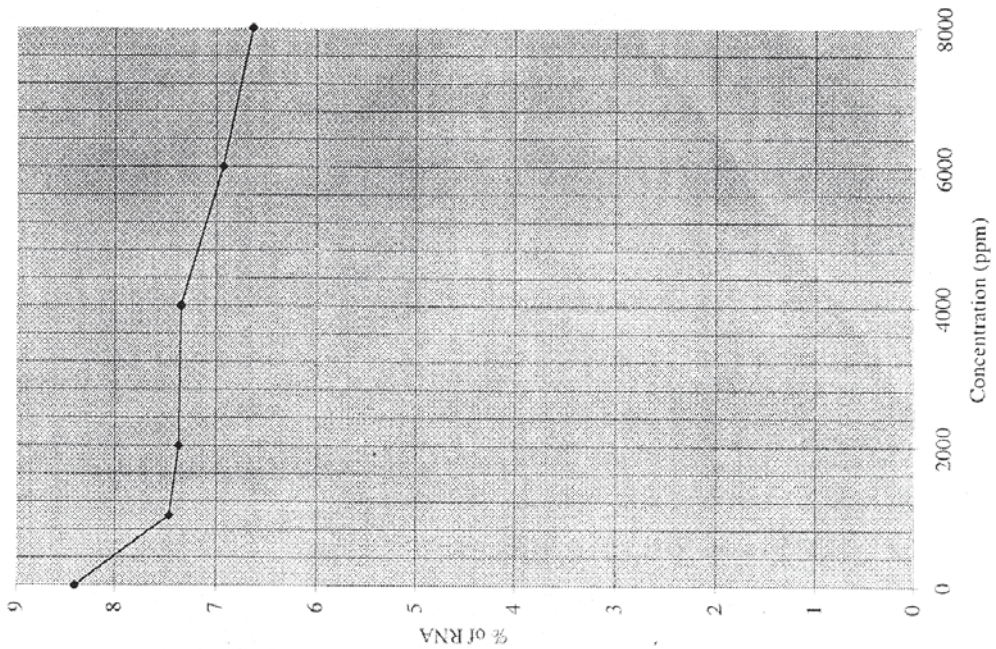

Fig. - 4: Graph showing RNA percentage per seedling at different concentrations of goal (oxyfluorfen)

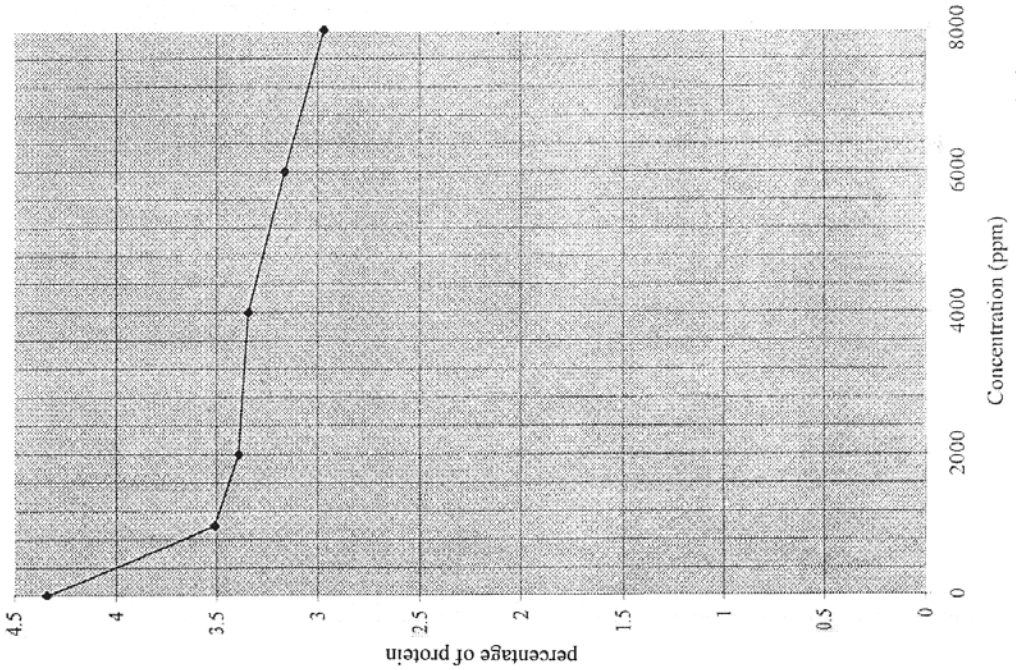

Fig. - 5: Graph showing percentage of protein contents per seedling at differnet concentrations of goal (oxyfluorfen)

was calculated from the standard graph of calf-thymus DNA (Fig.1). The DNA per seedling in a sample was calculated using the formula.

$$\text{DNA per seedling} = \frac{\text{Total DNA}}{\text{Total no. of seedling per sample}}$$

RNA

The sample of PCA extract containing RNA used for dilution which was ranging from 0.25, 0.50, 0.75 and 1.0 ml were taken in different test tube and volume was made upto 2 ml with distilled water. In blank reading was prepared by using 2 ml distilled water. In each tube added freshly prepared 2 ml of orcinol reagent. They allow to cool and read the absorbance at 600 nm on spectrophotometer. The RNA content in sample was calculated by using standard graph of yeast RNA (Fig. - 2). The RNA per seedling in a sample was calculated by the formula:

$$\text{RNA per seedling} = \frac{\text{Total RNA}}{\text{Total no. of seedling per sample}}$$

Extraction and estimation of total proteins

The treated and untreated seedlings of each concentration was dried for 24 hours in oven at 60°C for 24 hours. The dried weighted samples of each concentration was taken in Kjeldahl's flask. About 30 ml of concentrations sulphuric acid, 1 gm of copper sulphate and 5 gm of potassium sulphate were added in flask. The flask was than heated gently in an inclined position. The heating was continued till the brown colour of liquid first produced, then it was disappeared and left behind clear contents. The Kjeldahl's flask then allow to cool and contents diluted with some distilled water and carefully transferred into one litter round bottom flask. The flask then fitted with a dropping funnel and the Kjeldahl's trap. A vertical condenser was attached to the Kjeldahl's trap and the order end of

condensed was dipped in a beaker containing 25 ml of 0.1 N sulphric acid solution containing two drops of an indicator methyl red. The liquid in the round bottom flask was then heated and liberated ammonia distillate into beaker containing sulphric acid when no more ammonia passes over (tested the distillate with red litmus paper). The beaker containing sulphuric acid solution was removed and titrated with standard alkali (0.1 NaOH) solution and reading were noted. The standardization of normality of alkali and acid determined by titration of PHT (Potassium hydrogen thallate).

The percentage of nitrogen in the seedling was calculated by using formula:
N_2 % =[Normality of standard acid x volume of acid] – [Normality of alkali x volume of alkali] x 14 x 1000 x 100/ weight of sample.

The total protein of the sample was calculated from the obtained N_2 percentage.
Total protein = N2 Percentage x 6.25

Similarly the percentage of protein per seedling was calculated as follows:

$$\text{Protein per seedling} = \frac{\text{Total protein}}{\text{Total no. of seedling per sample}}$$

RESULTS AND DISCUSSION

After application of goal (Oxyfluorfen) the nucleic acid and protein contens of the seedlings showed gradual decrease as concentration of herbicide increased. The percentage of DNA per seedling was decreased with an increasing concentrations of herbicide. The DNA percentage was 5.9263 x 10^{-3}, 5.6009 x 10^{-3}, 5.2904 x 10^{-3}, 5.1241 x 10^{-3}, and 4.9865 x 10^{-3} at 1000, 2000, 4000, 6000 and 8000 ppm, respectively as against 9.9903 x 10^{-3} in control (Table - 1, Fig. -3).

The RNA percentage per seedling decreased gradually with an increase in the concentrations of herbicide. Thus the percentage of RNA seedling was 7.4577 x 10^{-3}, 7.3635 x 10^{-3}, 7.3393 x 10^{-3}, 6.9302 x 10^{-3}, and 6.6381 x 10^{-3} at 1000, 2000, 4000, 6000 and 8000 ppm respectively (Table - 2, Fig. -4). The percentage of DNA and RNA per seedling was gradually decreased as the concentration of herbicide increased. Similarly, decrease in the protein contents of seedling was observed. Thus, the protein percentage per seedling was 3.51, 3.39, 3.34, 3.16 and 2.97 at 1000, 2000, 4000, 6000 and 8000 ppm respectively as against 4.34 in the control (Table -3, Fig. -5).

Goal (Oxyfluorfen) affected the nucleic acids and protein content of the seedlings. The percentage of DNA in seedlings decreased with an increase in concentrations of herbicide similarly. RNA and protein contents were also decreased gradually as the concentrations increased.

Few reports are available in literature on effect of oxyfluorfen on nucleic acids and protein contents. Marawaba (1986) on potato reported decreased the protein content due to the application of oxyfluorfen. Gopal, (1993) on *Medicago sativa* reported decreased in percentage of nucleic acid and protein contents. Recently, Kulkarni (1998) on *Crotolaria medicaginea* var. *luxurians* reported decrease in percentage of DNA and RNA contents as compared to control due to application of this herbicide. The foregoing observations indicate that the herbicide probably blocked DNA, RNA and protein contents.

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