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EFFECT OF HERBICIDE GLYPHOSATE ON NUCLEIC ACIDS AND PROTEIN IN THE SEEDLINGS OF of *Sida acuta* Burm. F.

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ABSTRACT—

The effect of herbicide on macromolecular contents of seedlings was studied at the concentrations from 100 to 40,000 ppm of glyphosate. The DNA, RNA and protein contents of seedlings decreased gradually with the increased concentration of herbicide. The DNA, RNA and protein content of control seedlings was observed 0.9 x 10^{-4}, 1.3 x 10^{-4} and 2.1 x 10^{-4}, respectively.

Glyphosate treated seedlings, the percentage of DNA, RNA and protein content per seedlings at 100, 1000, 5000, 10,000, 20,000 and 40,000 ppm were 0.7 x 10^{-4}, 0.8 x 10^{-4}, 0.6 x 10^{-4}, 0.6 x 10^{-4}, 0.5 x 10^{-4} and 0.4 x 10^{-4}, respectively, 1.2 x 10^{-4}, 1 x 10^{-4}, 0.8 x 10^{-4}, 0.7 x 10^{-4}, 0.5 x 10^{-4} and 0.3 x 10^{-4}, respectively, 1.95 x 10^{-4}, 1.90 x 10^{-4}, 1.83 x 10^{-4}, 1.75 x 10^{-4}, 1.50 x 10^{-4} and 1.20 x 10^{-4}, respectively.

Thus, from the above study, it is concluded that glyphosate was effective in inhibiting macromolecular synthesis.

KEY WORDS: *Herbicide, Glyphosate, DNA, RNA and Protein*

INTRODUCTION :

MATERIALS AND METHODS

The seeds of *Sida acuta* Burm.f. were treated with different concentration of glyphosate for 24 hours in test tube. After treatment, seeds were washed thoroughly with distilled water and kept for germination in petridishes with double layered moistened filter paper in laboratory conditions. Seeds soaked in distilled water for 24 hours were used as control. The treated and untreated seeds were allowed to grow for six days.

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

EXTRACTION AND ESTIMATION OF NUCLEIC ACIDS:

The weighed samples were first homogenized in 5 ml of 10% perchloric acid (PCA) at 0^0C in a glass pestal and mortar and centrifuged the homogenate at 0^0C to 4^0C for 5 minutes. Discarded the extracts and resuspended the residue in cold 5% PCA and centrifuged again for 5 minutes. The supernatent was discarded and residue was washed sequentially with 70% alcohol, 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 to 5^0C for 18 hours. The solution was then centrifuged and supernatent was collected. The residue was resuspended with cold 2N PCA where centrifuged and two supernatents were combined and made the volume upto 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 70^0C for 20 minutes and the solution was centrifuged. The suprnatent was collected and the residue was resuspended again with hot 1N PCA and centrifuged. Both supernatents then combined and made volume to 20 ml by adding distilled water, which was comprised DNA fraction and was used for extraction of DNA.

The total RNA and DNA extractes were estimated by measuring absorbance at 660 and 595 nm, respectively and read the optical density with the help of spectrophotometer. The DNA and RNA contents in samples were calculated by using standard graph of calf-thymus DNA and standard graph of Yeast RNA, respectively. It is represented graphically. The DNA and RNA per seedling in a sample were calculated by using the following formula.

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

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

EXTRACTION AND ESTIMATION OF TOTAL PROTEINS:

The treated and untreated (control) seedlings of each concentration were dried in oven at 40-60^0C for 24 hours. The weighed dried samples (500 mg) of each concentration were taken in Kjeldahl's flask. About 30 ml of concentrated sulphuric acid together with potassium sulphate and copper sulphate (5:1) were added. The flask then heated gently in an inclined position. The heating was continuing till the brown colour of liquid produced, and then it disappeared and left behind clear contents. The Kjeldahl's flask then allowed cooling and contents were diluted with some distilled water and carefully transferred into one litre round bottom flask. An excess of 40% sodium hydroxide solution was poured down the sides of flask and it was fitted with Kjeldahl trap and a water condenser. The lower end of condenser dipped in 25 ml of 0.1 N sulphuric acid solution containing 2 drops of phenolphthalein indicator. The liquid in round bottom flask was then heated and liberated ammonia got distilled into sulphuric acid contained in a beaker. When no more ammonia passes over (tested the distillate with red litmus paper), the receiver was removed. The excess of acid was then determined by titration with N/10 sodium hydroxide solution using phenolphthalein as indicator and noticed the burette reading. The standardisation of normality of alkali and acid was determined by titration of PHT (potassium hydrogen thallate). The content of nitrogen in the seedling was calculated by using formula.

$$N_2\% = \left[\begin{matrix}\text{Normality of}\\ \text{standard acid}\end{matrix}\right] \times \left[\begin{matrix}\text{Volume}\\ \text{of acid}\end{matrix}\right] - \left[\begin{matrix}\text{Normality of}\\ \text{alkali}\end{matrix}\right] \times \left[\begin{matrix}\text{Volume}\\ \text{of alkali}\end{matrix}\right] \times \frac{14}{1000} \times \frac{100}{\text{Weight of sample (500mg)}}$$

From the obtained nitrogen content, the total protein of sample was calculated as follows:

$$\text{Total protein} = \text{Nitrogen content} \times 6.25$$

Similarly, the content of protein per seedling was calculated as follows:

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

RESULTS AND DISCUSSION

After treatment with glyphosate nucleic acid and protein contents were found to be decreased as the concentration of herbicide increased.

The content of DNA per seedling at 100, 1000, 5000, 10,000, 20,000 and 40,000 ppm was 0.7 x 10^{-4}, 0.8 x 10^{-4}, 0.6 x 10^{-4}, 0.6 x 10^{-4}, 0.5 x 10^{-4} and 0.4 x 10^{-4}, respectively as against in control was 0.9 x 10^{-4} while in percentage of RNA per seedling at 100, 1000, 5000, 10,000, 20,000 and 40,000 ppm, was 1.2 x 10^{-4}, 1.0 x 10^{-4}, 0.8 x 10^{-4}, 0.7 x 10^{-4}, 0.5 x 10^{-4} and 0.3 x 10^{-4}, respectively as against 1.3 x 10^{-4} of control. The content of DNA and RNA per seedling was decreased when concentration of herbicide increased.

The gradual decrease of protein content was observed as the concentration of herbicide increases. At 100, 1000, 5000, 10,000, 20,000 and 40,000 ppm the content of protein per seedling was 1.95 x 10^{-4}, 1.90 x 10^{-4}, 1.83 x 10^{-4}, 1.75 x 10^{-4}, 1.50 x 10^{-4} and 1.12 x 10^{-4}, respectively, whereas it was 2.10 x 10^{-4} in control.

The herbicide affected nucleic acids and protein contents of seedlings. The DNA per seedling decreased with an increase in the concentration of herbicide. Similarly, RNA contents also reduced along with increasing concentration of herbicide. Protein content also decreases per seedling with increase in concentration of herbicide. Thus, it may be concluded that herbicide was effective to reduce DNA, RNA and protein content in *Sida acuta* Burm.f. with gradual increase in concentrations.

This herbicide was effective on macromolecular synthesis of seedlings of *Sida acuta* Burm.f. The gradual decrease in nucleic acids and protein content was observed from 100 to 40,000 ppm. Many earlier workers noticed the inhibition of DNA and RNA synthesis following glyphosate treatment. Pillai *et al.* (1978) in soybean root reported that inhibit the uptake and incarporation of 14^{C}-thymidine into DNA, 14^{C}-uridine into RNA and 14^{C}-leucine into protein. Brecke (1976) in bean reported that decreased incorporation of 14^{C}-uracil into DNA. Tymonko (1978) reported that at 10^{-3} M inhibited RNA content by 33 % in enzymatically isolated soybean leaf cells. Cole *et al.* (1983), noticed inhibition of macromolecular synthesis (DNA, RNA and protein) in single node buds of *Agrophyrum repens* partly due to inhibition of 14^{0}-precursor uptake. Further, they concluded that glyphosate may partially exert its influence on bud development by regulating entry of assimilates into bud. Jain (1993) in *Chenopodim album*, Bobde (1993) in *Crotalaria juncea*, Kulkarni (1998) in *Crotalaria medicaginea*, Tulankar (1998) in *Amaranthus lividis*, Kamble (1999) in *Hibiscus cannabinus*, Dudhe (2002) in *Hyptis suaveoluns* and recently Kamble Sanjay (2006) in *Hibiscus cannabinus* also noticed decrease in the percentage of DNA and RNA in seedlings after the treatment of 2,4-D.

In the present study, the total protein content of seedling decreased as concentrations of herbicide increased. Pillai *et al*. (1978) on *Glycine max* reported decrease in protein content by glyphosate treatment. Tymonko and Foy (1978) in soybean. Cole *et al.* (1980), in *Glycine max* observed percentage of protein decreased by this herbicide. Jain (1993) in *Chenopodium album*, Kulkarni (1998) in *Crotalaria medicaginea* var. *luxurians*, Dudhe (2002) in *Hyptis suaveoluns* and recently Kamble Sanjay (2006) in *Hibiscus cannabinus* reported decrease in protein content due to application of glyphosate.

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