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EFFECT OF HERBICIDE STOMP ON NUCLEIC ACIDS ANS PROTEIN IN THE SEEDLINGS OF *Sida acuta* Burm. F.

Kamble S. I.
Associate Professor and Head, Department and Convener
Department of Seed Technology
Phulsing Naik Mahavidyalaya Pusad, Dist. Yavatmal,
Maharashtra State INDIA.

ABSTRACT :

The effect of herbicide stomp on macromolecular contents of seedlings was studied at the concentrations from 10 to 100 ppm. 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.

Following stomp treatment, DNA, RNA and proteins content per seedling at 100, 1000, 5000, 10,000, 20,000 and 40,000 ppm were 0.8 x 10^{-4}, 0.7 x 10^{-4}, 0.6 x 10^{-4}, 0.5 x 10^{-4}, 0.5 x 10^{-4} and 0.4 x 10^{-4}, respectively, 1.3 x 10^{-4}, 1.2 x 10^{-4}, 0.9 x 10^{-4}, 0.8 x 10^{-4}, 0.6 x 10^{-4} and 0.3 x 10^{-4}, respectively, 2 x 10^{-4}, 1.98 x 10^{-4}, 1.60 x 10^{-4}, 1.60 x 10^{-4}, 1.16 x 10^{-4} and 1.13 x 10^{-4}, respectively.

KEYWORDS : *Herbicide, Stomp, DNA, RNA and Protein*

INTRODUCTION :

MATERIALS AND METHODS

The seeds of *Sida acuta* Burm.f. were treated with different concentration of stomp herbicide 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 and 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 extracts 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.

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

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 ANS DISCUSSION

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

In stomp treated seedlings, there was also a gradual decrease in the amount of DNA per seedling at 100, 1000, 5000, 10,000, 20,000 and 40,000 ppm was 0.8 x 10^{-4}, 0.7 x 10^{-4}, 0.6 x 10^{-4}, 0.5 x 10^{-4}, 0.5 x 10^{-4} and 0.4 x 10^{-4}, respectively as against control 0.9 x 10^{-4} (Table-7, Fig.-110), while the content of RNA per seedling at 100, 1000, 5000, 10,000, 20,000 and 40,000 ppm was 1.3 x 10^{-4}, 1.2 x 10^{-4}, 0.9 x 10^{-4}, 0.8 x 10^{-4}, 0.6 x 10^{-4} and 0.3 x 10^{-4}, respectively, while in control it was 1.3 x 10^{-4} (Table-8, Fig.-110).

The amount of protein content per seedling also found to be decreased with the increase in concentration of herbicide stomp. It was 2.00 x 10^{-4}, 1.98 x 10^{-4}, 1.60 x 10^{-4}, 1.60 x 10^{-4}, 1.16 x 10^{-4} and 1.13 x 10^{-4} at 100, 1000, 5000, 10,000, 20,000 and 40,000 ppm, respectively as against 2.10 x 10^{-4} in control (Table-9, Fig-113).

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

Stomp was effective on nucleic acids and protein content of seedling which showed some variations. The DNA and RNA content per seedling decreased gradually with an increase in the concentrations.

It indicates that reduction in amount of DNA and RNA due to mitotic activity was accompanied with a depressive action. From 100 to 40,000 ppm, (Tables-7, 8 and Fig.-109) gradual reduction in nucleic acids contents was observed with increase in the rate of cell division and seedling growth. These effects may be well related to the observed stomp induced promotion or inhibition of DNA and RNA synthesis at higher concentrations. Here, it may concluded that mitotic inhibition by this herbicide has been attributed to blocking of mitotic cycle during interphase which may results from a prolonged G_2 period or to the inhibition of nucleic acids synthesis. Similar result also reported by Jain (1993) in *Chenopodium album* and Dudhe (2002) in *Hyptis suaveoluns*.

As the research is concerned, the literature about the effect of stomp on nucleic acids and proteins, there are several reports that indicate variations in nucleic acids and protein contents by different herbicides other than stomp of dinitroaniline group is considered. Schultz *et al.* (1968) found that RNA and DNA contents of root tips from intact maize seedlings germinated in trifluralin was decreased about 18 and 31 %, respectively. However, they remained similar to control in shoots. In the same experiment, they also found that after 72 and 92 hours treatment synthesis of nucleic acids in the shoots were markedly stimulated and suggested that the increased activity in the DNA may be due to a high guanine-cytosine found in the mohoring plants' tissues.

Lighnowski (1969) reported that 10^{-5} M trifluralin had no significant effect on RNA content of wheat root tips. Gruenhagen and Moreland (1971) and Moreland *et al.* (1969) reported that trifluralin at 2 x 10^{-4} M did not affect RNA synthesis or ATP content of excised soybean hypocotyls. Penner and Early (1972) reported that the trifluralin and butralin applied at 10^{-5} M to corn seedlings reduced RNA synthesis. Dukes and Biswas (1967) reported increase in nucleic acid contents with trifluralin at low concentration in sweet potato and peanut plants, but at higher concentrations, there was gradual decrease in the nucleic acid content.

Since, DNA and RNA is concerned in protein synthesis, the decrease in amount of nucleic acid will also affect the protein content of seedling. The gradual decrease in protein content of seedling was observed in the present study. In general, protein is a major storage reserve in many plants' seeds. This storage protein is hydrolysed during seed germination by proteolytic enzyme that is essential for seedling growth. Ashton *et al.* (1968) reported trifluralin at 1.5 x 10^{-6} M inhibited proteolytic activity 50% compared to control in squash seedlings. Tsay and Ashton (1971) observed that trifluralin at 1.5 x 10^{-6} M inhibited dipeptidase activity of squash cotyledon 15% compared to control. Ashton *et al.* (1977) also reported 80% inhibition of protein synthesis at 5 x 10^{-5} treatment of trifluralin in *Phaseolus vulgaris*. It is clear that, dinitroaniline herbicides have direct effects on enzymes, which is responsible for protein synthesis. Similar effect may occur in the present study and resulted into decrease in amount of protein content of the seedling after stomp treatment. It suggest that the herbicide action of degradation of reserve materials on the control during germination may be one of the factors responsible for greater susceptibility of seedlings to

herbicides. Jain (1993) and Dudhe (2002) in *Chenopodium album* and *Hyptis suaveoluns*, respectively, observed gradual decrease in protein content of seedlings.

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