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EFFECT OF HERBICIDE 2,4-D ON NUCLEIC ACIDS AND PROTEIN IN THE SEEDLINGS OF Sida acuta Burm. F.

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ABSTRACT

The effect of 2,4-D on macromolecular contents of seedlings was studied at the concentrations from 10 to 100 ppm of 2,4-D. The DNA, RNA and protein contents of seedlings decreased gradually with the increased concentration of herbicides. 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.

In 2,4-D treated seedlings, the DNA per seedling at 10, 20, 40, 60, 80, 100 ppm was 0.5 x 10^{-4}, 0.4 x 10^{-4}, 0.3 x 10^{-4}, 0.3 x 10^{-4}, 0.02 x 10^{-4} and 0.02 x 10^{-4}, respectively. RNA per seedling was 1.2 x 10^{-4}, 1 x 10^{-4}, 0.8 x 10^{-4}, 0.5 x 10^{-4}, 0.07 x 10^{-4} and 0.03 x 10^{-4}, while protein contents were 1.60 x 10^{-4}, 1.58 x 10^{-4}, 1.46 x 10^{-4}, 1.31 x 10^{-4}, 1.18 x 10^{-4} and 0.54 x 10^{-4}, respectively.

Thus, from the above study, it is concluded that 2,4-D was effective in inhibiting macromolecular synthesis.

KEYWORDS : *Herbicide, 2,4-D, DNA, RNA and Protein*

INTRODUCTION:

MATERIALS AND METHODS

The seeds of *Sida acuta* Burm.f. were treated with different concentration of 2,4-D, 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 homogenised 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 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 2,4-D nucleic acid and protein contents were found to be decreased as the concentration of herbicide increased.

The DNA per seedling in control was 0.9 x 10^{-4} whereas in 2,4-D treated seedling at 10, 20, 40, 60, 80 and 100 ppm was 0.5 x 10^{-4}, 0.4 x 10^{-4}, 0.3 x 10^{-4}, 0.3 x 10^{-4}, 0.02 x 10^{-4} and 0.02 x 10^{-4}, respectively. The content of DNA per seedling after 2,4-D treatment decreased suddenly from control to 100 ppm and that was 0.9 x 10^{-4} to 0.02 x 10^{-4}, respectively. RNA per seedling at 10, 20, 40, 60, 80 and 100 ppm was 1.2 x 10^{-4}, 1.0 x 10^{-4}, 0.8 x 10^{-4}, 0.5 x 10^{-4}, 0.07 x 10^{-4} and 0.03 x 10^{-4}, respectively as against control 1.3 x 10^{-4}). The gradual decrease of RNA content was observed as concentration of herbicide increases.

Protein per seedling decreased with an increase in concentration of this herbicide. It was 1.60 x 10^{-4}, 1.58 x 10^{-4}, 1.46 x 10^{-4}, 1.31 x 10^{-4}, 1.18 x 10^{-4} and 0.50 x 10^{-4} at 10, 20, 40, 60, 80 and 100 ppm, respectively as against in control content of protein was 2.10 x $10^{-4)}$.

Above result of DNA, RNA and protein of 2,4-D decrease with increase concentration as compared to standard.

This 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.

The decrease in DNA content was due to arrest of DNA synthesis as evident from the cytological studies. Most of the meristematic cells were found to be in an interphase. Similar results were obtained by earlier workers. Malhotra and Hanson (1966) observed that 2,4-D enhanced synthesis of DNA in susceptible plants like soybean and cucumber but not in resistant plants such as wheat and barley. Key *et al.* (1966) analysed the 2,4-D induced changes in RNA, DNA and protein at different zones of hypocotyls of soybean and found that DNA synthesis was stopped in hypocotyls zone. Chand and Roy (1981) in *Nigella sativa*, Nath *et al.* (1991) reported that there was consistently lowering of DNA content with increasing dose of 2,4-D in two kharif varieties of *Zea mays,* Jain (1993) in *Chenopodim album*, Gopal (1993) in *Medicago sativa,* 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*, Taduwadi (2002) in *Cleome viscosa* and recently Kamble Sanjay (2006) in *Hibiscus cannabinus* also noticed decrease in the percentage of DNA in seedlings after the treatment of 2,4-D.

In 2,4-D treated seedling the RNA content decreased gradually with the increase in concentrations. Many workers have noticed decrease in RNA content of seedling after 2,4-D treatment. Key and Shannon *et*

al. (1964) observed decrease in RNA in soybean seedlings with 2,4-D treatment. Key (1963) in corn and Shannon *et al.* (1964) in mesocotyl tissue of *Zea mays* found inhibition of RNA synthesis with 2,4-D treatment. Fites (1965) reported decrease in DNA and RNA content in soybean tissue following 2,4-D treatment. Moreland *et al.* (1969) noticed effect of 22 different weedicides on RNA and protein synthesis in maize mesocotyl and soybean hypocotyls and found that 14 of herbicides inhibited RNA and protein synthesis *in vitro*. Chen and Kozlowski (1972) observed progressive decrease in RNA levels in wheat by treatment with 2,4-D, 2,4,5-T, Dicamba and Picloram. Chand and Roy (1981) in *Nigella sativa*, Fedtke (1982) reported inhibition of RNA synthesis in corn plant roots by 2,4-D in dark and light conditions. Srinivasu and Bakale (1989a) noticed that at 10 and 50 ppm of 2,4-D, the RNA content increased gradually and later on at higher doses, it decreased with the increasing concentrations in *Parthenium hysterophorus*. Jain (1993) in *Chenopodium album*, Gopal (1993) in *Medicago sativa,* 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*, Taduwadi (2002) in *Cleome viscosa* and recently Kamble Sanjay (2006) in *Hibiscus cannabinus* reported inhibition of RNA by 2,4-D.

In the present study, protein contents of the 2,4-D treated seedlings decreased with the increase in the concentration. Similar results were stated by Stahler and Whitehad (1950) in sugarbeet, Freiberg and Clark (1952) and Key *et al.* (1966) in soybean. They studied the protein synthesis in different apicals zone of hypocotyl of soybean following 2,4-D treatment reported that 2,4-D blocked protein synthesis in apical zone of hypocotyls. Kolhe (1979) noted decrease in the protein content of *Solanum surattense*, following 2,4-D treatment, Chand and Roy (1981) in *Nigella sativa*, Sairam *et al.* (1986) reported that accumulation of 2,4-D in oat crop resulted in an increase protein contents at initial stage, however, at later it reduced drastically. Srinivasu and Bakale (1989a) also noticed gradual decrease in the protein content of *Parthenium hysterophorus* seedlings with an increase concentration of 2,4-D. Sen *et al.* (1987) reported that protein content in *Cyperus rotundus* increased initially but decreased after application of 2,4-D. Jain (1993) in *Chenopodim album*, Gopal (1993) in *Medicago sativa,* 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*, Taduwadi (2002) in *Cleome viscosa* and recently Kamble Sanjay (2006) in *Hibiscus cannabinus* also found gradual decrease in the protein contents per seedling with the increase in concentration of 2,4-D.

The foregoing discussion indicates that 2,4-D, decrease nucleic acids and protein content of seedlings of *Sida acuta* Burm. f.

REFERENCES

- Bobde, S. N. 1993. Comparative effects of herbicides on Crotalaria juncea L. Ph. D. Thesis, Nagpur Uni., Nagpur.
- Chand, S. and Roy, S. C. 1981. Effect of herbicides 2,4-dinitrophenol on mitosis, DNA, RNA and protein synthesis in Nigella sativa L. Biologia Plantarum. 20(3):192-202.
- Chen, W. C. and Kozlowski, T. T. 1972. Some histological effects of direct contact of Pinus resinosa seeds and young seedlings with 2,4,5-T. Weed Res. 12:229-223.
- Dudhe, S. 2002. Cytomorphological effect on weed by Hyptis suaveoluns L. Ph. D. Thesis, Nagpur University, Nagpur.
- Fedtke, C. 1982. Biochemistry and Physiology of herbicides Action. Springer Vellau. Bulletin. pp. 202.

- Fites, R. C. 1965. Alternation of the nucleic acid metabolism of soybean seedlings induced by 2,4-dichlorophenoxyacetic acid. Quoted from : Mechanism of action of herbicides. (Moreland, D. E.). Ann. Rev. Pl. Physiol. 18:365-386.
- Freiberg, S. R. and Clark, H. F. 1952. Effect of 2,4-D upon the nitrogen metabolism and water relations of soybean plants growth at different nitrogen levels. Bot. Gaz. 113:322-333.
- Gopal, K. R. 1993. Herbicidal effect on cytomorphology of weed Medicago sativa Linn. Ph. D. Thesis, Nag. Univ., Nagpur.
- Jain, S. B. 1993. Cytomorphological effects of weedicides on weed Chenopodium album. Ph. D. Thesis, Nagpur Univ., Nagpur.
- Kamble, S. I. 1999. Effect of herbicides on cytomorphology and macromolecular synthesis of Hibiscus cannabinus Linn. Ph. D. Thesis, Nagpur Univ., Nagpur.
- Kamble, Sanjay I. 2006. Effect of herbicide 2,4-D on DNA, RNA and protein contents of seedlings of Hibiscus cannabinus Linn. Biosciences, Biotechnology Research Asia. 3(1a):203-208.
- Key, J. L. 1963. 2,4-D induced changes in ribonucleic acid metabolism in excised corn mesocotyl tissue. Weeds. 11:177-181.
- Key, J. L. and Shannon, J. C. 1964. Enhancement by auxin on ribonucleic acid synthesis in excised soybean hypocotyls tissue. Pl. Physiol. 39:360-364.
- Key, J. L., Lin, C. Y., Gifford, F. M. (jr) and Dengler, R. 1966. Relation of 2,4-D induced growth aberrations to changes in nucleic acid metabolism in soybean seedlings. Bot. Gaz. 127(2-3):87-89. Kolhe, R. R. 1979. Effect of herbicides on the cytomorphology of farm weeds. Ph. D. Thesis, Nagpur Univ., Nagpur.
- Kulkarni, G. B. 1998. Effect of agro-chemicals on Crotalaria medicaginea var. luxurians. Ph.D. Thesis, Dr. Babasaheb Marathwada Univ., Aurangabad.
- Malhotra, S. S. and Hanson, J. B. 1966. Nucleic acid synthesis in seedlings treated with theauxin herbicide tordon (4-amino-3, 5, 6- trichloropicolinic acid). Pl. Physiol. Lancaster, 41 : suppl. 6.
- Moreland, D. E., Malhotra, S. S., Gruehagen, R. D. and Shokraii, E. H. 1969. Effect of herbicides on RNA and protein synthesis. Weed Sci. 17:556-563.
- Nath, R., Singh, S. and Bhambota, V. K. 1991. Biochemical response of maize varities to 2,4-D. Mysore J. Agric. Sci. 25:275-279.
- Sairam, R. K., Tomer, P. S. and Harika, A. S. 1986. Effect of subherbicidal level of 2,4- D, Isoproturan and Atrazine on the nitrogen metabolism and growth of oats (Avena sativa Linn.) Indian J. Plant Physiol. 29(3):237-242.
- Sen, D. N., Kasera, P. K. and Sundaramoorthy, S. 1987. Biology of weed and their control by some new herbicides in Indian arid zone. 11th Conf. Asian-Pacific Weed Sci. Soc. Taiwan Abstr. pp. 18.
- Srinivasu, T. and Bakale, V. L. 1989a. 2,4-D induced changes in DNA, RNA and crude proteins of Parthenium. Proc. Conf Cytol. and Genet. 2:237-241.
- Stahler, L. M. and Whitehad, E. J. 1950. The effect of 2,4-D on potasium nitrate levels in leaves of sugarbeets. Science. 112:749-775.
- Taduwadi, S. 2002. Effect of agrochemicals on cytomorphology of Cleome viscosa. Ph. D. Thesis, Nagpur University, Nagpur.
- Tulankar, 1998. Cytomorphological effects of herbicides on Amaranthus lividis L. Ph.D. Thesis. Nagpur University, Nagpur.