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Effects of Herbicide 2,4-Dinitrophenol on Mitosis, DNA, RNA and Protein Synthesis in *Nigella sativa* L.

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Abstract. Effect of 2,4-dinitrophenol (DNP) was studied on *Nigella sativa* to note the changes in mitosis, DNA, RNA and protein synthesis. The chemical affected division frequency considerably and chromosomal abnormalities like sticky bridge, fragmentation, micronuclei *etc.* were recorded. By using precursors of nucleic acid and protein synthesis, it was found that DNP also inhibited DNA, RNA and protein synthesis. The decrease in division frequency can be correlated with the DNA synthesis.

The substituted phenolic herbicides have been in use for over fifty years. The commonly used herbicides of this group are 2,4-dinitrophenol (DNP), pentachlorophenol, 4,6-dinitro-*o*-cresol and 2-sec-butyl-4,6-dinitrophenol. Some of them are highly toxic chemicals and cause dermatitis, weakness in the nervous system, weight lose, profuse sweating and blindness after prolonged administration. But most of the works were done in mammalian systems. However, the cytological effect of different phenolic herbicides on plants is very small.

The present work deals with the effect of herbicide 2,4-dinitrophenol on mitosis of *Nigella sativa* L. The effect on DNA, RNA and protein synthesis was also studied using labelled precursors of nucleic acid.

MATERIAL AND METHODS

The seeds of *Nigella sativa* were sown in moist sawdust. Seeds bearing roots (about 1.0—1.5 cm long) were taken out of the pots and thoroughly washed in tap water followed by washing in distilled water. Different concentrations of the chemical 0.1; 0.05; 0.01; 0.005 and 0.001% were taken in small tubes and seeds bearing roots were placed on the side of the tubes with the help of fine porous paper at 24 °C in such a way that the roots remained in direct contact with the solution. A control set for each exper-

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TABLE 1
Effect of 2,4-dinitrophenol on *Nigella sativa* root tip cells

Treatment [h]	Recovery [h]	Percentage of dividing cells					
		Control	Chemical concentration [%]				
			0.1	0.05	0.01	0.005	0.001
2	—	7.2	6.3	6.7	7.0	7.1	7.4
	24	7.8	6.1	6.3	6.6	7.3	7.8
	48	7.0	5.2	5.8	6.1	6.7	6.9
	72	6.2	4.1	4.4	4.8	5.3	5.8
4	—	6.9	5.1	5.4	5.6	6.0	6.4
	24	7.6	5.7	6.0	6.4	6.2	6.7
	48	6.7	5.5	6.1	6.6	6.9	7.2
	72	5.8	4.8	5.2	5.4	6.1	6.3
6	—	6.3	4.6	5.0	5.3	5.8	6.1
	24	7.1	5.1	5.6	5.8	6.6	7.0
	48	6.2	4.9	5.4	6.0	6.3	7.2
	72	5.4	4.4	4.6	4.8	5.4	5.7
24	—	6.5	4.3	4.8	5.0	5.3	5.8
	24	6.2	5.3	5.6	6.0	6.4	6.7
	48	5.7	4.6	5.2	5.8	6.0	6.2
	72	4.8	4.2	4.4	5.0	5.2	5.4

iment was kept in distilled water. After direct treatment in the chemical for definite periods (2; 4; 6 and 24 h), a few root tips were excised and fixed in acetic acid : ethyl alcohol (1 : 2) mixture. The remaining seeds with roots were transferred into Knop's nutrient solution for recovery from 24 to 72 h. After fixation for one hour, the root tips were treated with 45% acetic acid for 2—3 min followed by staining in 2% acetic-orcein — 1M HCl mixture (9 : 1) for one hour. Root tips 1—2 mm long were squashed in 45% acetic acid, slides were prepared and observed under the microscope. A considerable number of cells (about 1000 cells) were observed in each case. The average of three repetitions were taken into consideration.

For observing the effect of the herbicide on DNA, RNA and protein metabolism, the chemical treatment was as described above. After the desired period of herbicide treatment, the roots were washed 3—4 times in distilled water, followed by treatment with radioactive isotopes (supplied by Bhabha Atomic Research Centre, Trombay, Bombay — 85) for 1 h. 5 μCi ml^{-1} of ^3H thymidine (specific activity 8900 mCi $mmol^{-1}$), 10 μCi ml^{-1} of ^3H uridine (specific activity 2800 mCi $mmol^{-1}$) and 10 μCi ml^{-1} of ^3H lysine (specific activity 1200 mCi $mmol^{-1}$) were used for the study of the effects of these herbicides on DNA, RNA and protein synthesis, respectively.

After treatment with labelled precursors for 1 h, the roots were washed continuously in tap-water for 1 h to remove the traces of the isotope left on the surface of the roots. These were fixed in acetic acid : ethyl alcohol

1 μCi = 37 kBq.

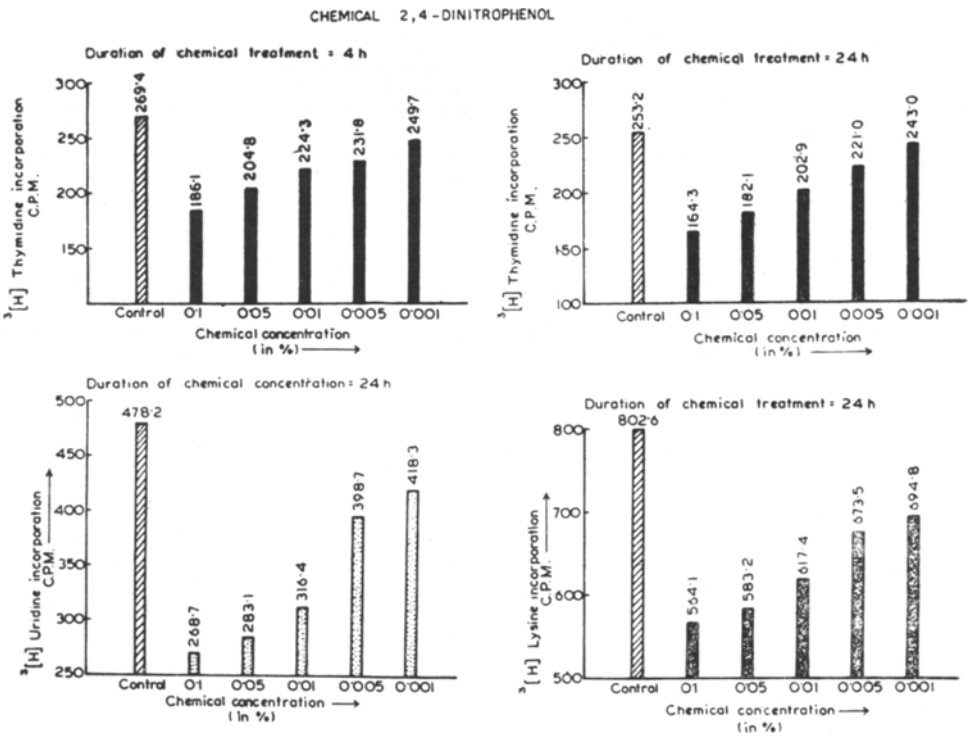

Fig. 1. Histograms showing effect of 2,4-dinitrophenol on DNA, RNA and protein synthesis at various concentrations.

(1 : 3) mixture overnight. The root tips (about 2 mm long) were excised and homogenized in a Sorvall Omni Mixer. Perfectly homogenized material of 100 root tips were placed on 2.3 cm filter paper discs of Whatman 3 MM paper. The discs were impaled on stainless steel pins for handling purposes.

The discs were picked up with the help of forceps and dropped into ice-cold trichloroacetic acid (10%) for 30 min. Then the discs were washed in 5% TCA three times followed by two rinses in ether : ethanol (1 : 1). A final washing was done in anhydrous ether. Discs were dried in a dust free room. Counting was performed by transferring each disc to a scintillation vial containing 10 ml of scintillation fluid (a solution containing 4 g of POP and 40 mg of POPOP in 1 l of toluene). Counts were taken in a liquid scintillation counter. Blank readings were obtained in all cases and subtracted from each reading of the sample. Results are expressed in the table as counts per min (c.p.m.).

RESULTS

In all the concentrations studied, division frequency was found lower than the control (Table 1). The division frequency was proportionately increased as the concentration becomes low, but it always remained lower than the control. Stickiness and clumping of chromosomes were of common

occurrence. Most of the anaphase showed sticky bridge formation even after the short duration of treatment. Sticky bridges were also observed after 72 h of recovery in some cases. Micronuclei usually one in number, were observed after direct treatment with the chemical. Diplochromatids and in some cases lagging of chromosomes were found. One to many strands were involved in the formation of sticky bridges. Multipolar spindle formation was also observed.

By using precursors of nucleic acid and protein, it was found that DNP inhibited DNA, RNA and protein synthesis. Inhibition was greater in 0.1% and 0.05% concentrations (Fig. 1).

DISCUSSION

The effects of phenol herbicides on the percentage of chromosomal anomalies induced and on mitotic index can be correlated with the concentration of the chemicals as observed by other workers on *Allium* and other plant species (LEVAN and TJIO 1948, VAN'T HOF and WILSON 1962, SHARMA and GHOSH 1965, SIKKA and SHARMA 1976). In plants most of the phenolic herbicides inhibit oxidative phosphorylation (WEINBACH 1956, GAUR and BEEVERS 1959, KANDLER 1960, MITCHELL 1961). The inhibition of oxidative phosphorylation results in inhibition of cell division (KIHLMAN 1966). This is because the development of the mitotic process requires energy. Most of the energy required by the cell is provided by oxidative phosphorylation in mitochondria in the form of ATP. EPEL (1963) has shown that the rate of mitosis in the sea urchin egg is closely related to the ATP level. The decrease in division frequency has also been noted by AMER and ALI (1969). They studied the effect of *o*- and *p*-nitro-phenol in *Vicia faba* and found that mitotic index was low at 0.025% to 0.1% concentrations. The delay in the appearance of interphase nuclei in mitosis and changes in the duration of the mitotic cycle in plants have been noted by HINDMARCH (1951) and VAN'T HOF and WILSON (1962) and TAKATORI (1965).

In the present investigation, the inhibition of DNA, RNA and protein synthesis with 2,4-dinitrophenol treatment have been noted by using tritium labelled precursors. The effect was more drastic at higher concentrations. Thus, the decrease in division frequency by the phenolic herbicides has some correlation with the inhibition of DNA synthesis. This inhibition in DNA, RNA and protein synthesis by DNP might be due to the reduction of oxidative phosphorylation in plants resulting in the lowering of ATP levels. ASHTON and CRAFTS (1973) stated that all herbicides that reduced ATP levels are strong inhibitors of RNA and protein synthesis.

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BOOK REVIEW

FRITSCHEN, L. J., GAY, L. W.: ENVIRONMENTAL INSTRUMENTATION. — REICHLE, D. E. (ed.): Springer Advanced Texts in Life Sciences. Springer Verlag, New York—Heidelberg—Berlin 1979. Pp. 216. DM 42.—, US $ 23.60.

Rapid advances in the knowledge of the relationships between physiological processes and environmental variables *i.e.* temperature, irradiance, humidity, wind and pressure has raised requirements on precise measurements and registration of meteorological and micrometeorological factors. This interest was also increased in consequence of man's possible alteration of environment and by the possibility of modelling biological processes facilitated by intensive computer development. The testing of biological models has demonstrated the need for more, different and precise data on environmental variables. Technological development, as *e.g.* the introduction of integrated circuits, brought about progressive recording methods for data acquisition systems coupled with computers, but the basic principles of measurement and sensors have remained in use for a relatively long time. Books on the measurement of environmental variables are out of date and new information has been scattered in numerals sources. This book was written in an attempt to bring together the information on sensors, instruments, and devices for electronic recording of environmental variables and to fill up the gap in the literature on this object.

The introductory two chapters provide an overview of measurement fundamentals and a review of the principles of energy transfer and d.c. circuits. The following chapters were devoted to measurement of "Temperature", "Soil heat flux", "Radiation", "Humidity and moisture", "Wind speed and direction" and "Pressure". The final chapter "Data acquisition concepts" deals with conversion of physical quantities (temperature, radiation, heat flux density *etc.*) into electrical signals with in turn are recorded and interpreted, and with the transducers for the conversion of information from one domain to another. All chapters are complemented by a list of references, unfortunately most of the papers cited are older than ten years and thus they do not sufficiently cover the progress achieved in the last decade. The book is closed by a detailed Subject index.

Specialists in forestry, agronomy, ecology, plant physiology and related branches now have a valuable source of information that should stimulate the application of environmental variable measurement. This well-arranged self-contained book with many illustrative figures and useful tables will certainly find its way to the library of many students and researchers.

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