

Genotoxic Effects of 2,4 - Dichlorophenoxyacetic Acid (2,4-D) on *Allium Cepa* L.

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Abstract

In the present study, the cytogenetic effects of herbicide substance 2,4-D were evaluated on *Allium cepa* L. The roots of the plants were treated with 2 ppm, 4 ppm and 6 ppm concentrations of for 3, 4 and 5 h. The Root tips after having grown to a certain length (2-3 cm) were cut and stained with acetocarmine stained squash prepared. All the concentrations of 2,4-D used significantly induced abnormalities such as c-Mitosis, bridges, laggards, multipolar cells compared to control.

KEYWORDS: Chromosome bridge, genotoxicity, herbicides and 2,4-D herbicides.

Introduction:

Herbicides are compounds designed to control the undesirable plants (weed) that may interfere with the growing of commercial crops (Blair et al. 1990). 2,4-dichlorophenoxyacetic acid (2,4-D) is a herbicide commonly used in Indian agriculture. The residues of 2,4-D are present in air, water, soil and edibles. It constitutes a real hazard for human and animal health as numerous accidents of poisoning deaths caused by this herbicide have been reported (Bukowska, 2006).

The systemic and selective herbicide 2,4 - dichlorophenoxyacetic acid (2,4-D) is applied mainly to eliminate broad leaf species, where it initiates the action of natural plant hormone indole acetic acid when used in small amounts; besides, in high concentrations it induces chromosome abnormalities (Kallak and Javekylg 1971; Bushraet al. 2002). Unfortunately, the herbicide does not target only weeds. It can cause low growth rates, reproductive problems, changes in behavior, or death in non-targeted species.

The indiscriminate use of pesticides and herbicides to control the pest and weed in agriculture as well as the increase of pollution in ecosystems due to industrial development, need to evaluate the toxicity of these chemicals. They can be converting into mutagenic or carcinogenic agents by vegetables, which are the first living beings to absorb the nutrients from polluted environments and acting as the toxic agents' vectors to humans (Marcanoet al. 2004). Different chemicals have been studied in vitro and in vivo, which are used for the detection and monitoring of a wide variety of environmental toxic chemicals with mutagenic and carcinogenic potential (Ashby et al.1988). Root tip systems of various plants have been widely used for determining the harmful effects of mutagens (Khilman 1975; Ma and Grant 1982; Rank and Neilsen 1994). Chromosomal aberrations are indicators of genetic damage induced by pesticides (Reddi and Reddi 1985). It has been suggested that 2,4-D or other phenoxy herbicides may causes Non-Hodgking's Lymphoma (NHL) and other cancers (Holland et al. 2002). It has been reported that 2,4-D causes chromosomal damage in root cells of plants. Recently certain researches have been suggested that the chemicals that are commonly added to pesticides may change the activity of pesticides (Holland et al. 2002). The *Allium cepa* is a very good model plant for the assessment of chromosome damage both in mitosis and meiosis.

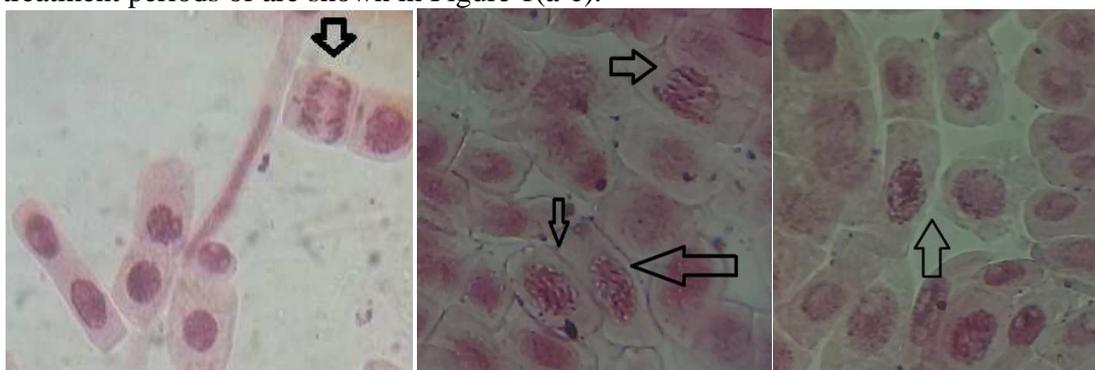
The present study was carried out to determine the effect of 2,4-D on the process of mitosis on *Allium cepa* L. at different concentrations.

Materials and methods:

The clean and healthy bulb of *Allium cepa* L. was selected. Before starting to the experiments, dry scales of bulbs were removed, and 2ppm, 4ppm and 6ppm concentrations of herbicide were used. The solutions were prepared in distilled water. *Allium cepa* L. roots were treated with different concentrations for 3, 4, and 5 h. Controls were treated with distilled water for the same periods. For the microscopic observations, root tips were fixed in Carnoy's fluid and hydrolyzed in 1 N HCl for 5 min in an oven at 60 °C. This was followed by the preparation of crushed material with aceto Carmine stain.

Results:

All of the concentrations of 2,4-D used in the present study induced abnormalities during mitotic division as compared to control in *Allium cepa* L. These abnormalities were c-Metaphase, chromosomes tickiness, anaphase bridges, laggard chromosomes, multipolar anaphase, and fragments. The highest abnormality was observed in root tips in 6ppm concentration with 6 h treatment period. Distributions of abnormalities according to stages were different. The most frequent abnormalities were seen at metaphase and at this stage the predominant type of abnormality was c-Mitosis followed by fragmentation. The examples of chromosomal abnormalities induced by different concentrations and treatment periods of are shown in Figure 1(a-c).



a) Anaphase bridge b) C-metaphase and multipolarity c) Fragmentation

Discussion:

Several epidemiological studies previously reported an association between occupational 2,4-D exposure and NHL and other cancers (Holland et al. 2002). The plant tests in some ways are more sensitive than both the micro assay and the Ames test. It can even detect some carcinogenic substances that are negative to the Ames test (Rank and Nielsen 1994). It has been suggested that plant chromosome analysis may be of some help in cancer research (Levan 1951). Many authors reported the cytogenetic effects of 2,4-D that it induced chromosome abnormalities in the meiosis of *Vicia faba* (Khilman 1975) and barley plants (Khalatkar and Bharagava 1985). 2,4-D herbicide induced mitotic chromosome aberrations in the roots of some plants (Kumari and Vaidyanath 1989; Bodade 1996). It also induced sister chromatid exchanges in cultured immature embryos of wheat species (Pijnaker and Ferwerda 1994). Furthermore, 2,4-D used in commercial products may have different peculiarity from pure 2,4-D (Holland et al. 2002). 2,4-D

significantly induced mitotic chromosome aberrations at all concentrations in *Allium cepa* L. The most common abnormality observed in the present study was C-Mitosis. Gomurgen (2000) and Bushra et al. (2002) have also demonstrated that 2,4-D herbicides induce c-Mitosis. Large number of c-Mitosis indicates that 2,4-D acts as potent spindle inhibitor due to which all anaphase chromosomes lie on the metaphase plate instead of moving towards their respective poles (Bushra et al. 2002). Also, lagging chromosomes and multipolar anaphases were seen in this study. Lagging chromosomes may be attributed to the failure of spindle apparatus to organize in a normal way (Patil and Bhat 1992). Multipolar anaphases are indicating the inhibition of cytokinesis (Gomurgen 2000). As a result, the present study shows that commercial herbicide formula of 2,4-D is genotoxic as it induced chromosome abnormalities in *Allium cepa* L.

References:

1. Ashby J., F.J. De Serres, M.D. Shelby, G.H. Margolin, M. Ishidate and G.C. Becking (Eds). (1988) :Evaluation of short term tests for carcinogens, report of the international programme on chemical safety's Collaborative Study on in vitro assay. Vols I /II, Cambridge University Press, Cambridge.
2. Bodade S.N. (1996): The mitotic effect of herbicide 2,4-D in *Crotalaria Juncea* Linn, Adv. Plant Sci., 9:73-74.
3. Bukowska B. (2006): Toxicity of 2,4-dichlorophenoxyacetic Acid– Molecular Mechanisms. Polish J. Environ. Stud. Vol. 15(3): 365-374.
4. Bushra A., F.M. Abdul, A.M. Niamat, and W. Ahmad (2002) Clastogenicity of 2,4-Dichlorophenoxy acetic acid in vitro and in vivo. Mutation Research, 521: 165-178.
5. Holland N.T., P. Duramad, N. Rothman, L.W. Figgs, A. Blair, A. Hubbard and M.T. Smith, (2002) :Micronucleus frequency and proliferation in human lymphocytes after exposure to herbicide 2,4-Dichlorophenoxy acetic acid in vitro and in vivo. Mutation Research, 521: 165-178.
6. Kallak H. and L. Javeky (1971): Cytogenetic effects of 2,4-D on pea callus in culture. Acta Biol. (Budapest), 22: 67-73.
7. Khalatkar A.S. and Y.R. Bharagava (1985): Mutagenic effects of 2,4-dichlorophenoxy acetic acid alone and with ethyl methane sulphonate in *Hordeum vulgare* L., Environmental Pollution Series A- Ecological and Biological., 38: 9-17.
8. Khilman B.A. (1975): Root tips of *Vicia faba* for the study of the induction of the chromosomal aberrations. Mutation Research, 31: 401-412.
9. Kumari T.S. and K. Vaidyanath (1989): Testing of genotoxic effects of 2,4-Dichlorophenoxy acetic acid (2,4-D) using multiple genetic assay systems of plants, Mutat. Res., 226: 235-238.
10. Levan A. (1951): Chemically induced chromosome reactions in *Allium cepa* and *Vicia faba*. Cold Spring Harbor Symp. Quant. Biol., 16: 233-243.
11. Ma T.H. and V.F. Grant (1982): The Tradescantia adventitious plants. Herbarist, 48: 36-44.

12. **Marcano L., I. Carruyo, A. Del Campo and X.A. Del Campo and X.Montiel, (2004):** Cytotoxicity and mode of action of Maleic hydrazide in root tips of *Allium cepa* L. *Environmental Research*, 94: 221-226.
13. **Patil B.C. and B.I. Bhat (1992):** A comparative study of MH and EMS in the induction of chromosomal aberrations on lateral root meristem in *Clitoria ternatea* L. *Cytologia*, 57: 259-264.
14. **Pijnaker L.P. and M.A. Ferwerda (1994):** Sister chromatid exchanges in cultured immature embryos of wheat species and regenerants. *Theoretical and Applied Genetics*, 89: 287-292.
15. **Rank J. and M.H. Nielsen (1994):** Evaluation of *Allium* Anaphase-telophase test in relation to genotoxicity screening of industrial wastewater. *Mutation Research*, 312: 17-27.
16. **Rank J., L. Lopez, H. Mette and J. Moretton (2002):** Genotoxicity of maleic hydrazide, acridine and DEHP in *Allium* root cells performed by two different laboratories'. *Hereditas*, 136: 13-18.
17. **Reddi T.V.V.S. and V.R. Reddi (1985):** Cytological effects of chemical mutagens in rice. *Cytologia*, 50:499-405.