J. Indian bot. Soc., 61 : 33—38, 1981.

# EFFECT OF TOK E-25 ON THE CYTOLOGY OF *MALVASTRUM COROMANDELIANUM* (L.) GARCKE.[1]

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## ABSTRACT

The seeds of *Malvastrum coromandelianum*, (Linn.) Garcke, were treated with concentrations ranging from 100 to 10,000 ppm of Tok E-25 for 24 hours. The lethal dose was found to be 10,000 ppm.

Cytological preparations of the treated root tips revealed various types of abnormalities such as bridges, laggards of chromosomes, fragments, precocious movement, clumping and grouping of chromosomes. Certain cells with two, three and even four nucleolated conditions were observed and some cells showed mega- and micronucleoli. The percentage of abnormalities for 1,000 and 9,000 ppm was 31.79 and 41.30, respectively. It was observed that with increasing concentration of the herbicide, a gradual decrease in the percentage of cells undergoing cell division occurred.

## INTRODUCTION

Many research workers (Crafts, 1961 ; Ashton and Harvey, 1971 ; Shrivastava *et al.* (1957) have been occupied during the last two decades in elucidating the effect of 2, 4-D and its derivatives for weed control. In recent years many selective herbicides such as 2, 4-D, 2, 4, 5-T, Picloram have been extensively used for eradication of obnoxious weeds such as *Ambrosia artemisiifolia* L., *Pastinaca sativa* L., *Solidago canadensis* L., *Solidago nemoralis* Ait, and *Vicia cracca* L. (Tomkins and Grant, 1976). These studies usually revealed only the gross responses of the plant to the chemicals. Basic work on the morphological, physiological and cyto logical responses of the plant is essential for a full understanding of the mode of action of these herbicides.

The present study was undertaken to investigate the cytological responses of *Malvastrum coromandelianum.* (Linn.), Garcke, an obnoxious weed of Maha rashtra to Tok E-25 (Nitrofen ; 2,4 dichlorophenyl-4-nitrophenyl ether).

## MATERIAL AND METHODS

The seeds of *Malvastrum coromandel ianum*, (Linn.), Garcke, were collected from the agricultural field of Punjabrao Krishi Vidyapeeth, Nagpur. The freshly collected seeds did not germinate under laboratory conditions due to seed coat dormancy, which was broken by chemical scarification. The seeds were then washed thoroughly with water and dried. The dried seeds were soaked for 24 hours in concentrations of Tok E-25 ranging from 100 to 10,000 ppm. After the pre soaking treatment, the seeds were washed thoroughly with water and kept for germ-ination on moist filter paper in petri-plates at room temperature.

The root tips from twodays old control seedlings and from seedlings of treated

1. Accepted for publication on February 26, 1981.

seeds were fixed in modified Carnoy's solution (absolute alcohol : acetic acid : chloroform, 1 : 1 : 1 by volume). The root tips were then washed with water and stored in 70% alcohol for cytological preparations.

The root tips were hydrolized in 1N HCl for 10 minutes at 60°C, washed with water and mordanted with iron alum for 3 minutes. After thorough washing with water they were stained with haematoxylin, squashed in 45% glacial acetic acid and permanent micropreparations were mounted in euparol.

The mitotic index was calculated as follows :—

$$\text{Mitotic Index} = \frac{\text{Total No. of cells undergoing mitosis}}{\text{Total No. of cells observed}} \times 100$$

## OBSERVATIONS

Normal mitosis was observed in control root tips. However, the treated root tips exhibited various types of abnormalities such as bridges (Fig. 1), precocious movement of chromosomes (Fig. 2), fragments (Fig. 3), clumping (Fig. 4) and laggards of chromosomes (Fig. 5). A few cells with both micro- and mega-nucleoli were observed (Fig. 6). Certain cells with two (Fig. 7), three (Fig. 8) and even four nucleolate (Fig. 9) conditions were of frequent occurrence. The presence of persistent nucleolus at metaphase was a noteworthy feature (Fig. 10).

All such cytological anamolies were scored right from 100 to 9000 ppm of Tok E-25, except the lethal dose (10,000 ppm). It was observed that as the concentration of herbicide increased, the percentage of cells undergoing division considerably decreased. The mitotic indices for the control, 100, 1000, 2000, 4000, 8000, and 9000 ppm treated plants varied from 25.69 to 1.98, (Table I). Ten thousand ppm was found as the lethal dose.

TABLE I

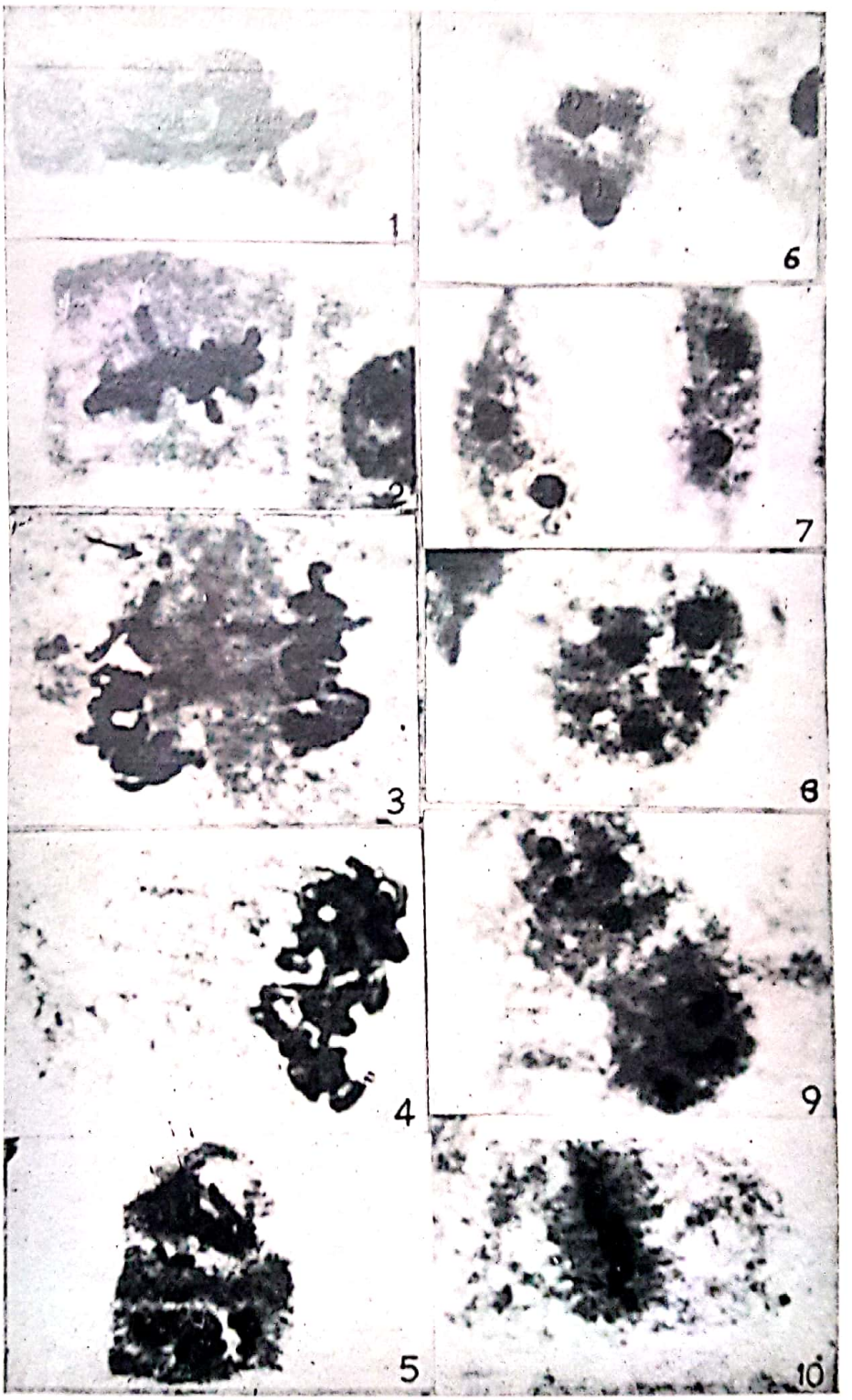
CHROMOSOMAL ABERRATIONS INDUCED DUE TO TREATMENT OF HERBICIDE TOK E-25 (NITROPEN) ON MITOTIC CELLS OF *MALVASTRUM COROMANDELIANUM*, (LINN). GARCKE

| Concentration in ppm. | Mitotic index | % of Abnormal cells. |
|---|---|---|
| Control | 25.69 | — |
| 100 | 23.76 | 17.44 |
| 200 | 19.37 | 20.91 |
| 400 | 17.88 | 24.81 |
| 600 | 10.37 | 28.72 |
| 800 | 9.16 | 29.93 |
| 1000 | 8.10 | 31.79 |
| 2000 | 7.06 | 33.51 |
| 4000 | 6.01 | 35.09 |
| 6000 | 5.05 | 39.70 |
| 8000 | 4.07 | 39.74 |
| 9000 | 1.98 | 41.30 |

Among the abnormalities the percentof age multipolar and dicentric bridges and precocious movements of chromosomes were greater at higher concentrations. The percentage of bridges and precocious movement was 15.21 and 17.39 at 9000 ppm, respectively (Table II).

## DISCUSSION

Tok E-25, induced various chromosomal aberrations as shown in Table 2. Ennis (1947) reported similar types of abnormalities in *Avena sativa*. due to carbamate, Muhling *et al.* (1960) in *Pisum sativum*, *Cynodon dactylon* following the




Figs. 1—10. Root tip cells of *Malvastrum coromandelianum*. Fig. 1. Bridge. Fig. 2. Precocious movement. Fig. 3. Fragment. Fig. 4. Clumping. Fig. 5. Laggard. Fig. 6. Mega and Micro nucleoli. Fig. 7. Two nucleoli. Fig. 8. Three nucleoli. Fig. 9. Four and three nucleoli. Fig. 10. Metaphase with persistant nucleolus. (Figs. 1 to 10 × 500).

TABLE II

PERCENTAGE OF DIFFERENT TYPES OF ABNORMALITIES

| Conc. in ppm | Bridges | Laggards | Precocious movement | Grouping | Clumping | Fragments | 2N | 3N | 4N | Mega N | Micro n |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| 100 .. | 4.20 | 1.34 | 5.36 | 0.33 | 1.34 | 2.01 | 1.67 | 0.67 | 1.00 | 5.36 | 4.02 |
| 200 | 5.00 | 1.42 | 5.71 | .. | 1.42 | 1.07 | 2.14 | 1.78 | 2.14 | 9.64 | 8.57 |
| 400 | 6.29 | 1.11 | 7.77 | .. | 1.85 | 1.85 | 1.85 | 1.48 | 2.59 | 10.37 | 8.14 |
| 600 | 7.63 | 1.81 | 9.45 | .. | 1.09 | 1.09 | 4.36 | 4.00 | 3.27 | 20.00 | 14.54 |
| 800 | 7.82 | 1.02 | 9.86 | 1.36 | 0.68 | 1.69 | 3.06 | 2.72 | 2.38 | 14.28 | 9.52 |
| 1000 | 9.73 | 2.05 | 10.76 | 1.53 | .. | 2.05 | 1.53 | 3.07 | 2.56 | 12.30 | 10.25 |
| 2000 | 10.43 | 1.09 | 11.53 | 1.09 | .. | 1.64 | 2.19 | 3.29 | 2.74 | 15.38 | 9.89 |
| 4000 | 11.25 | 0.66 | 12.58 | 1.32 | .. | 1.32 | 2.64 | 3.31 | 1.32 | 11.92 | 8.60 |
| 6000 | 12.59 | 1.57 | 13.38 | 0.78 | 1.57 | 0.78 | 1.57 | 3.93 | 0.78 | 11.81 | 10.23 |
| 8000 | 14.10 | .. | 15.38 | .. | 1.28 | 2.56 | .. | 3.84 | 2.56 | 12.82 | 8.97 |
| 9000 | 15.21 | .. | 17.39 | .. | 2.17 | .. | .. | 4.84 | 2.17 | 15.21 | 6.52 |

Abbreviation : 2N—Two Nucleolate ; 3N—Three Nucleolate ; 4N—Four Nucleolate ; Mega N—Mega Nucleolus ; Micro n—Micro Nucleolus.

action of phenols and Bingham (1968) in *Zea mays* and *Allium cepa* L. as a result of DCPA.

Fragmentation of chromosomes has also been reported in *Allium cepa* and *Vicia faba* after treatment of ionizing radiations and chemicals (Kihlman and Levan, 1951 ; Kihlman, 1955a). Insecticidal and pesticidal agents such as Gammaxane, DDT etc. are also known to produce chromosome breakages (D'Amato, 1950 ; Scholes, 1955). Amer and Ali (1968) showed that phenolic compounds induce breakages during meiosis and most of the breakages were of the chromatid type. Similar types of breakages were also noticed in the present study.

In addition, Tok E-25 also induced various anaphasic abnormalities such as multipolar and dicentric bridges due to stickiness. Unrau and Larter (1952) observed stickiness of chromosomes in large number of pollen mother cells of barley (*Hordeum vulgare*) and considered it to be due to changes in the chemical or electrostatic properties of nucleic acids of the chromosomes.

A noteworthy feature in the present study was the presence of persistant nucleolus in treatments of different concentrations at metaphase. However, it was not possible to give any explanation for its persistance in some and non-persistance in other cells. But however, Brown and Emery (1957) were of the

opinion that there was a correlation between the presence/absence of persistant nucleoli and cytological and anatomical characters in some grasses.

Several investigators, (Heitz, 1931 ; McClintock, 1934 ; Resende, 1937 ; Pathak, 1940) considered that the number of nucleoli corresponded to the number of SAT-chromosomes and the secondary constrictions. Sharp (1934) and Wilson and Morrison (1966) have stated that a single nucleolus is indicative of nucleolar fusion and proximity of organizers, while dual nucleoli result from comparatively widespread physical separation of the organizing regions. According to Mo gan (1971) dual nucleoli at somatic interphase could also be the consequence of limited production of nucleolar substance.

Gates (1942) considered that the number of nucleoli in the cells of species is as important phylogenetically as the number of chromosomes. The binucleolate condition in grape according to Raj and Seethaiah (1969) might be due to non-disjunction of a particular chromosome at meiosis. The spore receiving the extra chromosome has two nucleolar organizers and potentially two nucleoli.

In the present study the presence of micro- and meganucleoli were of frequent occurrence. Clay and Nath (1971) also reported one to three micronucleoli in quartets of *Cornus*, whereas Frankle (1937) recorded as many as four nucleoli in young tetrad cells of *Fritillaria pluriflora*.

From the foregoing, it could be concluded that Tok E-25 inducing various chromosomal aberrations, inhibition of mitosis which ultimately upsets the physiological set up of a cell and consequently leads to the death of the plant. Mitotic inhibition and chromosomal damage may be associated with secondary physiological changes (Gunckel, 1957).

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