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# EFFECTS OF DITHANE M-45 (A FUNGICIDE) ON ROOT MERISTEM OF *VIGNA MUNGO* (L.) HEPPER

**Susanta Kumar Maity***

**Abstract:** *The cytotoxic effects of Dithane M-45, a fungicide were investigated in the mitotic cell division in black gram (Vigna mungo L. Hepper) root tip cells. For this aim, with four different concentrations (0.1%, 0.2%, 0.3% and 0.4%) of Dithane M-45 solutions were used for ten hours. Root tips after having grown to a certain length (1.5 to 2 cm) were stained according to aceto-orcein squash procedure and the number of abnormal cells was counted in each phase of mitosis. The obtained results indicate that Dithane M-45 had the ability to cause production of a large number of mitotic abnormalities. These abnormalities appeared in varying degrees depending on the dose. Various abnormalities on chromosomes like lagging early anaphase, chromosomal bridges, c-metaphase, chromatin granulation etc were seen among mitotic divisions treated with Dithane M-45. Control roots did not reveal any abnormality.*

*Department of Botany, Chandernagore Govt. College, Chandernagore, Hooghly, West Bengal, India

## INTRODUCTION:

Black gram (*Vigna mungo* L. Hepper) is the important food grain legume and rich in protein. It is a pulse commonly used in Indian cuisine. In the last decades, the use of fungicides in agriculture for fungi diseases control has become crucial. Fungicides produce a diverse range of products with novel modes of action. The extensive use of these compounds in the agriculture system raises public concern because of the harmful potential of such substances in the environment and human health (Mendes *et al*., 2005). The extensive use of fungicides in plant protection against fungal disease generates long term residues in food and in the environment (Petit *et al*., 2008).

Fungicides may also influence to change plant genetic system due to their mutagenecity and carcinogenicity. Cytogenetic studies have been carried out to detect harmful effects of different pesticides on different plant species (Rank *et al*.,2002; Marcano *et al*., 2004). Mutation breeding has become increasingly popular in present times as an effective tool for crop improvement (Siddiqui and Khan, 1999). There are several studies aiming to explain and to understand the effects of fungicides in plant systems. Rayburn *et al*.,(1993) stated out that amount of nuclear DNA is decreased by the fungicide, captan and this fungicide has been mutagenic, carcinogenic and teratogenic effects on many organisms. Celik (2006) used two fungicides in his experiment, Derosol and Korsikol and examined by cytogenetic effects on barley root tip meristem cells. These two fungicides effect on chromosome fragments, bridge, stickiness and polar deviation. The present study has been carried out to investigate the influence of Dithane M-45 in *Vigna mungo* root tip cells during mitosis.

## MATERIALS AND METHODS:

Healthy and dry seeds of *Vigna mungo* were pre-soaked in tap water for 10 hours and then treated with Dithane M-45 at four different concentrations (0.1 to 0.4%) for ten hours. After treatments, the seeds were thoroughly washed with running tap water to remove the excess amount of fungicide from the seeds, if any. One set of seeds were kept untreated to act as control for comparison. Both the treated and controlled seeds were transferred to the Petridishes having the moist filter papers for germination. Fifty seeds were used from each dose and control. The Petridishes were kept at room temperature (28-30$^{o}$C) for 24 hours.

The root tips of germinated seeds (both experimental and control) having the length in 1.5-2 cm were excised and pretreated with aqueous para-dichlorobenzene for three hours,

washed with distilled water, fixed with glacial acetic acid:ethanol (3:1) solution and kept for 24 hours. After 24 hours the root tips were transferred to 70% ethanol and stored in a refrigerator. For examination, the root tips were first treated with 2% aceto-orcein and 1(N) HCl (9:1) and just warmed over a flame of sprit lamp. Slides were observed under compound microscope and 1000 cells were counted from each treatment. Mitotic index was expressed in terms of divided cells/total cells x100. All experiments were conducted with five replicates and average results were taken.

## RESULTS:

The Dithane M-45 fungicide applied to *Vigna mungo* seeds depending on concentrations was found to have effects on germination and cell division. Maximum number of seed germination was recorded in control (95%) where as it decreased in fungicide treated from 80% to 50% in 0.1 to 0.4% Dithane M-45. The mitotic index in control was observed to be maximum (14.23%) with no chromosomal anomalies. All concentrations of fungicide cause a decrease in MI when the different division stages were examined. The percentage of abnormal mitotic stages was seen to increase respectively with increasing fungicide concentration. The treated root tips showed various types of metaphasic and anaphasic aberrations at each dose of treatment. It also lowered MI value to 6.11 (14.23 in control) of root tip meristem cells of *Vigna mungo* with formation of various genotoxic abnormalities like condensed chromatin (Fig. 1&2), chromatin granulation, c-metaphase, chromosomal bridges (Fig 3), lagging Chromosome (Fig 4), sister chromatin distaining etc. Mitotic indices at different doses of Dithane M-45 have been shown in Table 1. At lowest concentration of Dithane M-45 (0.1%), the mitotic index is reduced to 11.37 and further increase in concentration, resulted in decline in mitotic index. When the seeds were treated with 0.4 % of Dithane M-45, the mitotic index was greatly reduced and found to be 6.11. The treated root tips showed various types of aberrations at each dose of treatment. Increase in concentration of Dithane M-45 significantly increased the mitotic inhibition and ensured the harmful effect on mitotic cycle. The most prevalent aberration caused by Dithane M-45was fragments at metaphase (32 %), bridges at anaphase (12 %) and stickiness at anaphase (32 %).

## DISCUSSION:

The germination rate of *Vigna mungo* seeds were reduced due to inhibitory effect of fungicide. Similar results have also been reported in other plant species like *Vicia faba* (Agarwal and Ansari, 2001), *Trigonella* sp. (Siddiqui *et al*., 2008). The several external factors for seed germination such as $O_2$ concentrations, light, moisture etc. are known to have influence germination (Isabella *et al*., 2000). Several other mutagenic agents and heavy metals have been shown to inhibit seed germination (Seregin and Kozhevnikova, 2006; Jabee *et al*., 2008).

The different concentrations of used fungicide effects on mitosis division in the root tip cells of *Vigna mungo* are shown in Table 1. The mitotic index is a reliable predictor of the cell proliferation in the tissue or organ. The decrease in the mitotic index in root tip meristems were observed in treated seeds when compared to the control and it decreased gradually in all the treatments with increasing concentrations of Dithane M-45. Similar results were found after treating the root tip cells of *Helianthus annuus* with copper chloride (Inceer *et al*., 2003). In the present study, the chromosomal aberrations induced by the fungicide Dithane M-45 included sticky metaphase, anaphase bridge and fragments may also be observed. Similar results have also been reported in *Trigonella* sp by Abbasi and Anis (2002); Jabee *et al*., (2008). Chromosomal stickiness is characterized by chromosomal clustering during any phase of the cell cycle. Stickiness and clumping may be caused by genetic and environmental factors. Several agents have been reported to cause chromosomal stickiness (Panneerselvam et al., 2012). The chromosome bridges were recorded at all the concentrations of the treated fungicide and it produced due to chromosomal breakage and joining of incorrect ends (Gill *et al*., 2000). However, Dithane M-45 fungicide has different effects on cell division mechanism. It may be concluded that as has been stated above, Dithane M-45 fungicide has harmful effects on the root tip meristem cells of *Vigna mungo* and it acts almost like a mutagen.

Table 1. Effect of *Dithane M-45* treatment on the MI and chromosomal aberrations in the root tip cells in *Vigna mungo*.

| Concentrations | % of seed germination | Total cells | Division cells | MI | Fragment | Bridge | Sticky chromosome |
|---|---|---|---|---|---|---|---|
| Control | 95 | 17.07 | 2.43 | 14.23 | - | - | - |
| 0.1 | 80 | 17.84 | 2.03 | 11.37 | 22 | 3 | 4 |
| 0.2 | 78 | 16.69 | 1.84 | 11.02 | 21 | 6 | - |
| 0.3 | 70 | 17.55 | 1.81 | 10.31 | 24 | 6 | 3 |
| 0.4 | 50 | 16.54 | 1.01 | 6.11 | 32 | 12 | 32 |

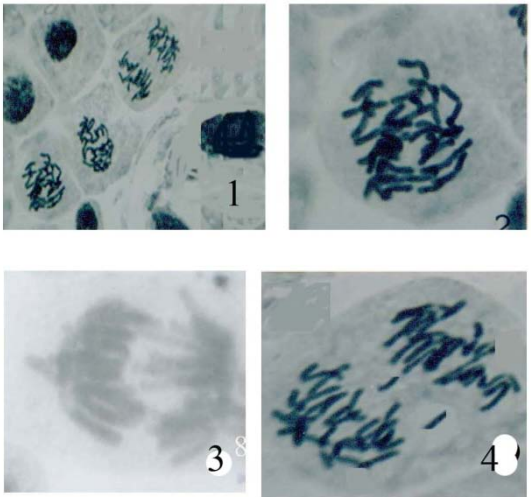


Figure captions:

Fig 1&2.Condensed chromatin,

Fig 3. Chromosome bridge,

Fig 4. Lagging chromatid

## REFERENCES:

a. Abbasi N and Anis M (2002). Clastogenic effect of chemical mutagens in *Trigonella foenum-graecum* L. J Cytol Genet 3 (NS): 109-114.

2. Agarwal R and Ansari MYK (2001). The effect of Aniline on root tip cells of *Vicia fava* L. J Cytol Genet 3 (NS):129-134.

3. Celik TS (2006). Cytogenetic effects of some fungicides on Barley root tip meristem cells, Pakistan Journal of Biological sciences. 9(13):2508-2511.

4. Gill RSA and Shaukat SS (2000). Genotoxic effects of *Dithane M-45* fungicide on root meristems of *Allium cepa* L. *in vitro.* Pakistan Journal of Biological sciences. 3(1):114-117.

5. Inceer H, Sema AYAZ, Osman B and Emine S (2003). Cytogenetic effects of copper chloride in root tip cells of *Helianthus annuus* L., Turk J Biol. 27:43-46.
6. Isabelle D, Leon KM and Koomneel M (2000). Influence of the testa on seed dormancy. Germination and longevity in *Arabidopsis.* Plant Physiol. 122:403-413.
7. Jabee F, Ansari M and Shahab D (2008). Studies on the effect of maleic hydrazide in root tip cells and pollen fertility in *Trigonella foenum- graecum* L. Turk J Biol. 32:337-344.
8. Marcano I, Carruyo I and Del Campo A (2004). Cytotoxicity and made of action of maleic hydrazide in root tips of *Allium cepa*, Environmental Research, 94:221-226.
9. Mendes CAC, Mendes GE, Cipullo JP and Burdmann EA (2005), "Acute intoxication due to ingestion of vegetables contaminated with aldicarb," Clinical Toxicology, 43(2), 117–118.
10. Panneerselvam N, Palanikumar L and Gopinathan S, "Chromosomal aberrations induced by Glycidol in *Allium cepa* L. root meristem cells." International Journal of Pharma Sciences and Research. 2012. 3:2, 300-304.
11. Petit AN, Fontaine F, Clément C and Vaillant-Gaveau N (2008), "Photosynthesis limitations of grapevine after treatment with the fungicide fludioxonil," Journal of Agricultural and Food Chemistry, 56(15), 6761–6767.
12. Rank J, Lopez LC and Nielsen MH (2002). Genotoxicity of maleic hydrazide, acridine and DEHP in *Allium cepa* root cells performed by two different laboratories. Hereditas, 135:13.
13. Rayburn LA, Wayne L and Pedersen L (1993). The fungicide *Dithane M-45* reduce nuclear DNA content in maize seedling. Pestic. Sci.,37:79-82.
14. Seregin V and Kozhevnikova AD (2006). Physiological role of nickel and its toxic effects on higher plants. Russian J plant Physiol.53:257-277.
15. Siddiqui BA and Khan S (1999). Breeding in crop plants, Kalyani Publishers, 20-34.
16. Siddiqui S, Meghvansi MK, Khan SS and ali NS (2008). Mutagenic effect of herbicide (maleic hydrazide) on seed germination and radical length of *Trigonella foenum-graecum* L. Ind J App Pure Biol.23:103-106.
17. Yuzbasioglu D (2003). Cytogenetic effects of fungicide afugan on the meristematic cells of *Allium cepa* L. Cytologia, 68:237-243.