

## EFFECT OF SOME FUNGICIDES ON CYTO-MORPHOLOGICAL CHANGES IN *ALLIUM CEPA* ROOTS

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Effect of various concentrations of some fungicides (Mancozeb, Bavistin and Sulphex) on cyto-morphological changes in roots was studied. Treatments with lower concentration of these fungicides reduced the time taken for root initiation. The number of roots per bulb increased by various treatments, but root length was reduced. Various treatments caused a significant reduction in the length, width and nuclear diameter of the root tip cells. The fungicide treatments also inhibited mitotic index. Percentage of anomalies at various stages of cell division increased with the increase in the concentration of fungicides. The anomalies observed were, nuclear vacuolation, chromosomal bridges, binucleate cells, scattered metaphase, disorientation of poles, laggard and multipolar anaphase.

**Keywords** : *Allium*; Cyto-morphology; Fungicides.

### Introduction

The use of chemicals to protect man and his crops from the attack of diseases has increased steadily since last two decades. The occurrence of pesticides and fungicides in the environment and subsequently in parts of food chain, resulting in the intake of these compounds by man and animal has already been noted since the early sixties<sup>1,2</sup>. The fungicides used to kill the plant pathogens lead to the destruction and development of various abnormalities in the plant cells and tissues. The present study was envisaged to determine the effect of some fungicides on cyto-morphological changes in *Allium cepa* roots.

### Materials and Methods

The *Allium cepa* var. Pusa Red was used as a test organism. Three concentrations (500, 1000, 1500 ppm) of three fungicides namely, Mancozeb (dithane M-45), Bavistin (methyl-3-benimidazole Carbamate) and Sulphex were taken in wide mouthed bottles. Five onion bulbs with their basal ends downwards were placed on each of these. For control, five bulbs were placed on bottles containing distilled water. Data on time taken for root initiation, number of roots/bulb and their length were collected. For cytological studies, root tips were fixed in Carnoy's

fluid for 24h and stored in 70% alcohol. These were hydrolysed in 1N HCl for 3 minutes and squashed in 1% acetocarmine. Data on cell length, width and diameter of nuclei and mitotic abnormalities were collected from 250 cells each in 10 different slides. Mitotic index was calculated as :

$$\text{Mitotic index} = \frac{\text{Total no. of cells in division}}{\text{Total no. of cells observed}} \times 100$$

### Results and Discussion

#### A. Morphological

(a) Root initiation : Time taken for root initiation in *Allium cepa* treated with lower concentrations of Mancozeb and Bavistin was reduced (Table 1). It is interesting to note that in bulbs treated with 500 ppm Bavistin roots developed within 24 hours as compared to 4 days taken by control bulbs. Similarly, bulbs treated with 500 and 1000 ppm Mancozeb took only 2 and 3 days respectively for root initiation. On the other hand, the bulbs treated with higher concentrations of Mancozeb (1500 ppm) and 1000 and 1500 ppm Bavistin took 5,6,8 days respectively for root initiation.

(b) Number of roots : Data in Table 1 shows that various treatments of both the fungicides enhanced the number of roots

on bulbs treated with them and the increase was directly proportional to the concentration. Maximum number of roots (72) were recorded on bulbs treated with 1500 ppm Mancozeb, while lowest number of roots (42) among treated bulbs were recorded in treatment with 500 ppm Bavistin as compared to only 33 roots on control bulbs.

(c) Root length : From the data in Table 1 it is clear that root length significantly decreased in all the treated bulbs. The smallest roots (1.74 cm) were recorded in the bulbs treated with 1500 ppm Mancozeb, while the largest roots (2.65 cm) were recorded in the treatment with 500 ppm Bavistin as compared to 11.3 cm long roots in control bulbs.

Thus, the results of the present study on root initiation and number of roots on bulbs treated with both Mancozeb and Bavistin clearly indicate that the effect of these fungicides is more or less similar to that of an auxin. It is supported by the fact that these treatments inhibited root length which may be due to their higher concentration<sup>4</sup>.

**Table 1** : Effect of Mancozeb and Bavistin on the time taken for root initiation, number of roots and length of *Allium cepa* roots. Data from five bulbs kept on each concentration.

Parameters	Concentration	Mancozeb	Bavistin
Time taken for root initiation (days)	Control	4	4
	500	2	1
	1000	3	6
	1500	5	8
No. of roots/bulb	Control	33±2	33±2
	500	58±3	42±3
	1000	63±4	57±3
	1500	72±3	68±4
Root length (cm)	Control	11.07 ±0.5	11.07±0.15
	500	1.803 ±0.15	2.65±0.1
	1000	1.78±0.1	2.547±0.1
	1500	1.74±0.05	1.949±0.05

± Standard deviation

## B. Cytological

(a) Cell length : Data in Table 2 shows that the cell length was gradually reduced with the increase in the concentration of all the three fungicides used. Maximum reduction in cell length (2.96  $\mu\text{M}$ ) was recorded in the root-tips on bulbs treated with 1500 ppm Sulphex and minimum reduction (4.64  $\mu\text{M}$ ) was recorded in the root tips on bulbs treated with 500 ppm Bavistin as compared to 5.68  $\mu\text{m}$  on long cells in roots on control bulbs. The reduction in cell length seems to be the cause of reduction in root length.

(b) Cell width : Treatments with all the concentrations of different fungicides reduced the cell width with the exception of the bulbs treated with all the three concentrations of Bavistin (Table 2). The roots grown in various concentrations of Bavistin showed an increase in cell width which increased with the increase in the concentration. The maximum width (3.38  $\mu\text{m}$ ) was recorded in roots of bulbs treated with 1500 ppm Bavistin as compared to that of control bulbs (3.62  $\mu\text{m}$ ).

(c) Nuclear diameter : Data in Table 2

**Table 2 :** Effect of fungicides on length, width and nuclear diameter of cells in root tips of *Allium cepa*. Data from 250 cells.

Parameters	Concentration (in ppm)	Mancozeb	Bavistin	Sulphex
Cell length(μm)	Control	5.68±0.5	5.68±0.15	5.68±0.5
	500	4.1±0.35	4.64±0.1	3.84±0.1
	1000	3.6±0.1	3.72±0.05	3.58±0.25
	1500	3.1±0.1	3.38±0.15	2.96±0.1
Cell width(μm)	Control	3.62±0.5	3.62±0.1	3.62±0.25
	500	2.76±0.5	2.74±0.15	3.14±0.1
	1000	2.72±0.25	2.34±0.1	2.74±0.05
	1500	2.24±0.1	2.38±0.1	2.34±0.1
Nuclear diameter (μm)	Control	2.78±0.1	2.78±0.1	2.78±0.15
	500	1.94±0.05	2.12±0.2	2.08±0.2
	1000	1.62±0.01	2.06±0.15	1.76±0.2
	1500	1.34±0.05	1.92±0.05	1.44±0.15

±Standard deviation

**Table 3.** Mitotic index and abnormalities in root tip cells treated with various concentrations of fungicides.

Fungicide Concentration (ppm)	No. of cells observed	No. of dividing cells	Mitotic Index (%)	Prophase		Metaphase		Ana-Telophase	
				Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Control	250	150	60	61	-	47	-	42	-
<b>Mancozeb</b>									
500	250	150	60	46	-	33	36	-	35
1000	250	130	52	23	18	18	26	18	20
1500	250	100	40	23	24	-	24	-	29
<b>Bavistin</b>									
500	250	110	44	24	-	22	23	19	22
1000	250	90	36	22	-	20	24	-	24
1500	250	60	24	12	-	12	18	-	18
<b>Sulphex</b>									
500	250	100	40	24	-	19	18	22	17
1000	250	80	32	15	-	14	21	15	15
1500	250	70	28	14	-	11	16	11	18

shows that the nuclear diameter of the treated bulbs decreased with the increase in the concentration. The smallest nuclei (1.34  $\mu\text{m}$ ) were recorded in the bulbs treated with 1500 ppm Mancozeb, while largest nuclei (2.12  $\mu\text{m}$ ) among various treatments were recorded in the bulbs treated with 500 ppm Bavistin as compared to that of nuclei of 2.78  $\mu\text{m}$  diameter in roots of control bulbs.

(d) Mitotic index : Data in Table 3 shows that these fungicides have inhibitory effect on cell division. A strong dosage effect is obvious from decline in the mitotic index values with the increase in the concentration. Maximum reduction in mitotic index (24%) was observed in the root cells treated with 1500 ppm of Bavistin, while, minimum reduction (60%) was recorded in the cells treated with 500 ppm Mancozeb.

(e) Abnormalities : Various types of mitotic abnormalities were recorded in the root tip cells treated with different concentrations of all the three fungicides. From the data in Table 3 it is clear that there were 18 and 24 abnormal prophase cells in 1000 and 1500 ppm Mancozeb treated roots cells respectively. On the other hand, various concentrations of Bavistin and Sulphex treated roots failed to show abnormal prophase. Cells in metaphase in roots treated with all the chemicals exhibited maximum abnormalities (abnormal metaphase, chromosome breakage and clumping). The maximum number of cells showing abnormal metaphase (26) were recorded in roots treated with 1000 ppm Mancozeb. The total number of abnormal metaphase cells in various Mancozeb treatments were 86, while, there were 65 abnormal metaphase cells in various Bavistin treatments. Similarly, various treatments with Sulphex induced abnormalities in 55 cell showing metaphase and maximum (21) being in 1000 ppm Sulphex

treatments. Various treatments also induced different kinds of abnormalities at ana- and telophase. These were disorientation of poles, laggards and bridges, multipolar anaphase and formation of binucleate cells. In various Mancozeb treatments 86 cells were recorded to exhibit abnormalities and maximum being (36) in 500 ppm Mancozeb treatments. There were 64 abnormal anaphase cells out of 250 cells observed in Bavistin treated roots, while 50 abnormal cells were recorded in various Sulphex treatments. 1500 ppm of both these chemicals (Bavistin and Sulphex) induced abnormalities in 18 cells in each treatment.

Similar mitodepressive and clastogenic effects of a piperidine and a fungicides, Topsin in *Allium cepa* roots was studied by Somashekar *et al*<sup>5,6</sup>. They have observed clumping and fragmentation of chromosomes and binucleate cells with vacuoles in the nuclei. Choudhary and Sajid<sup>7</sup> have also reported a large number of chromosomal abnormalities in the pea root-tips treated with Bavistin. Padmaja and Vidyavati<sup>8,9</sup> have studied the effects of two fungicides namely Fytolon and Bavistin on somatic cells of *Rhizochlonium* and found that these induce various nuclear and chromosomal abnormalities.

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