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Cytogenetic effects of fungicide Fludioxonil on root meristem cells of *Lens esculenta* L.

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Abstract

Lens esculenta is an important cereal and rich in protein content. Several fungicides used are often considered a quick, easy and inexpensive solution for controlling fungus. One of them fungicide is fludioxonil, its cytotoxic effects on root tip meristem cells of *Lens esculenta* were investigated. For this aim, four different concentrations (0.1%, 0.2%, 0.3% and 0.4%) of fludioxonil were given at seed level for 24 hours. Aceto-orcein squash preparation of root tips of the treated seeds showed the chromosomal aberrations. All the dosages of the fungicide fludioxonil caused various abnormalities like univalents, multivalents, laggards, bridges, micronuclei, stickiness etc. in different stages of mitosis division when compared with control. Chromosomal aberrations were found to be correlated with the concentration of fungicides.

Fludioxonil is a fungicide used to control fungal diseases, making it a useful seed treatment as well as a post-harvest treatment for fruit such as cereals, apples, blackberries, broad beans, combining peas, edible podded peas, pears, strawberries etc. In continuously developed agricultural systems depend on a wide variety of synthetically produced chemicals, including insecticides, fungicides, herbicides and other pesticides both in conventional methods and recently formed shree process. These used chemicals have hazardous effects in addition to their benefits in all aspects. The undesirable residues of fungicides in water, food and in environment may cause various types of deleterious effects on diverse living systems on the earth. Fungicides may also be effective to change plant genetic system due to their mutagenecity and carcinogenicity. There are several studies aiming to explain and to understand the effects of fungicides in plant systems. Rayburn[10] 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. Cytogenetic studies have been carried out to detect the harmful effect of different pesticides on different plant species[8,9,11]. Celik[3] used two fungicides in his experiment, Derosol and Korsikol and examined by cytogenetic effects on barley root tip meristem cells. This two

fungicides effect on chromosome fragments, bridge, stickiness and polar deviation. The present study was, therefore, undertaken to examine the effects of fludioxonil on mitotic activity and chromosomes in the root tip of *Lens esculenta*.

Healthy and dry seeds of *Lens esculenta* were pre-soaked tap water for 5 hours and then treated with fludioxonil at four different concentrations (0.1 to 0.4%) for 24 hours. The treated seeds were washed with running tap water for 15-20 minutes and taken on to the dryer filter paper. 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 (22-25ºC) for two days.

The root tips of germinated seeds [both experimental and control] having the length in 1.0-1.5 cm were excised and pretreated with 0.02% 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 upto 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.

The fludioxonil fungicide which is applied to *Lens esculenta* seeds depending on concentrations was found to have effects on germination and mitotic cell division. Maximum number of seed germination was recorded in control (90%) where as it decreased in fungicide treated from 80% to 50% in 0.1 to 0.4% fludioxonil. The mitotic index in control were observed to be maximum (16.33%) with no chromosomal anomalies. At all the treated concentrations of fungicide causes a decrease in MI when the different stage frequency were examined. The percentage of abnormal mitotic stages was seen to increase with higher concentrations of fungicide respectively. The treated root tips showed various types of aberrations at each dose of treatment. It also lowered MI value to 6.34 (16.33 in control) of root tip meristem cells of *Lens esculenta* with formation of various genotoxic abnormalities like condensed chromatin (Fig. 1), chromatin granulation (Fig 2), chromatin distaining, c-metaphase, chromosomal bridges (Fig 3), lagging Chromosome (Fig 4), sister chromatin distaining etc. Mitotic index were significantly decreased with increasing the concentrations of fungicide. Mitotic indices at different doses of fludioxonil have been shown in Table 1. At lowest concentration of fludioxonil (0.1%), the mitotic index is reduced to 10.83 and further increase in concentration, resulted in decline in mitotic index. When the seeds were treated with 0.4 % of fludioxonil, the mitotic index was greatly reduced and found to be 6.34. The treated root tips showed various types of metaphasic and anaphasic aberrations at each dose of treatment. Increase in concentration of fludioxonil significantly increased the mitotic inhibition and ensures the harmful effect on mitotic cycle. The most prevalent aberration caused by fludioxonil was fragments at

Table-1. Effect of fludioxonil treatment on the MI and chromosomal aberrations in the root tip cells in *Lens esculenta*

Concen-trations	% of seed germination	Total cells	Dividing cells	MI	Fragment	Bridge	Sticky chromosome
Control	90	19.32	8.41	16.33	-	-	-
0.1	80	17.44	8.21	10.83	19	3	3
0.2	75	19.69	5.35	11.02	21	5	2
0.3	56	19.52	4.85	10.31	24	11	3
0.4	50	19.24	1.76	6.34	30	12	35

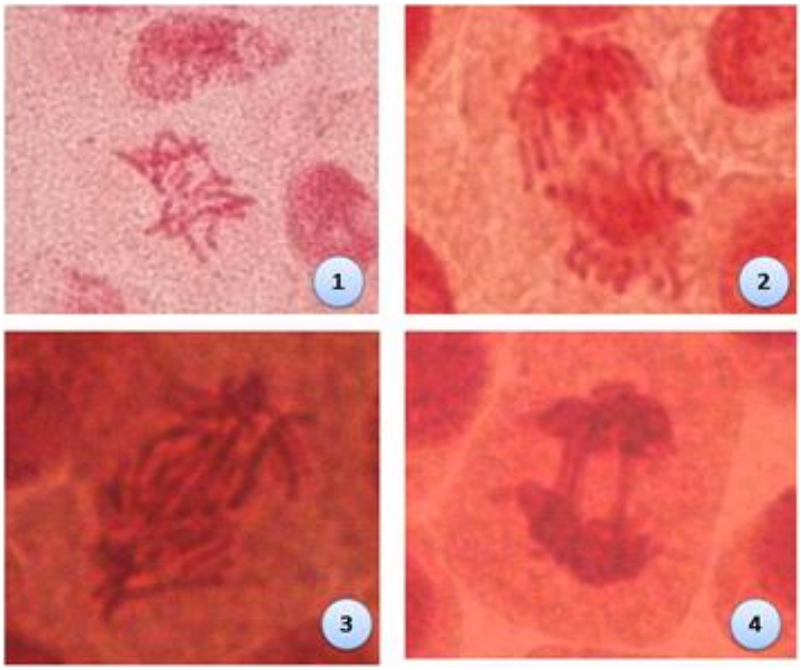

Figure captions:
Fig 1. Condensed chromatin, Fig 2. Chromatin granulation,
Fig 3. Chromosome bridge, Fig 4. Lagging Chromosome

metaphase (30 %), bridges at anaphase (12 %) and stickiness at anaphase (35 %) on 0.4% concentration.

The germination rate of *Lens esculenta* seeds were reduced due to inhibitory effect of fungicide. Similar results have also been reported in other plant species like *Vicia faba* [2] and *Trigonella* sp.[13]. Several external factors, mutagenic agents and heavy metals have been shown to inhibit seed germination in other plant species[6,7,12].

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 fludioxonil. Similar results were found after treating the root tip cells of *Helianthus annuus* with copper chloride[5]. In the present study, the chromosomal aberrations induced by the fungicide fludioxonil included sticky metaphase, anaphase bridge and fragments may also be observed. Similar results have also been reported in *Trigonella* sp. by Abbasi and Anis[1]; Gill[4]. 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. However, fludioxonil fungicide has different effects on cell division mechanism. It may be concluded that as has been stated above, fludioxonil fungicide has harmful effects on the root tip meristem cells of *Lens* and it acts like a mutagen.

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