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Studies on cytotoxic effects of cycloheximide on root meristem of *Allium sativum* L.

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Abstract
The cytotoxic effects of Cycloheximide, a widely used fungicide were investigated on meristematic cells of *A. sativum* L. The results showed that the fungicide is mitodepressive in nature. It also induced various types of chromosomal and nuclear aberrations such as chromatid separation, chromosomal bridge, laggards, polarity abolition, binucleate and multinucleate cells. It is concluded that cycloheximide not only inhibits mitotic activity but also shows adverse impact on chromosomal behavior of cells.
Key Words - Cytotoxic, Cycloheximide, Chromosomal aberrations.

Introduction:
Cycloheximide is an extensively used fungicide. Such biocides can easily get entered in the food chain and their concentration increases with successive trophic levels and indiscriminate use of such biocides may result in chronic poisoning.
The danger of long term consumption of biocides residue in food is far more serious than acute poisoning from the point of view of National Health. Sharma (1997) has referred the children born today have to start life with a body burden of pesticides which increases with age. According to Amer, Odette and Farah (1983) the phosphorothioate insecticide 'dursban' induces significant percentage of abnormal mitosis. The chromosomal abnormalities induced by 'endosulphan' during mitotic activity was studied by Aktac *et. al.*(1994). They also reported it's mitodepressive nature. It was reported by Priya *et. al.* (1996) that organophosphorous pesticide 'malathion' not only causes damage to the chromosome but also reduces the frequency of division. The present work is undertaken to evaluate mutagenic and cytotoxic potential of Cycloheximide on *A. sativum* L. root tip cells.

Material and Method:
Cycloheximide is an eukaryotic protein synthesis inhibitor produced by the bacterium *Streptomyces griseus*. It has been used as fungicide in agricultural applications. It is also known as Naramycin A, Hizarocin, Actidion etc. Its chemical formula is $C_{15}H_{22}NO_4$.

A. sativum L. was taken as test material. The cloves of the plant were grown in small trays containing sterile moist soil. After allowing roots to grow, the cloves were transferred to Petri dishes containing 100 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm of fungicide. The treatment of each concentration was given for the time intervals of 2, 4, and 6 hours. All the treatments were carried out at 22 - 25^0 C. After each treatment root tips were excised and fixed in acetic - alcohol (1 : 3) for 24 hours. The root tips were then transferred to 70% alcohol for preservation. The treated root tips were hydrolyzed in 1N HCl for 5 - 8 minutes and squashed in 2% acetocarmine for cytological studies. The slides were temporarily sealed, examined and microphotography of the selected aberrations was conducted using the method of Mousa (1982). Chromosomal aberrations and their percentages were also recorded.

Result and Discussion:

The results obtained by the treatment of cycloheximide on the root meristematic cells of *A. sativum* L. are given in the table -1. The cycloheximide a common fungicide has significantly suppressed the mitotic activity in root meristem. The increase in concentration as well as increase in duration of treatment, both are inversely proportional to the mitotic activity. The mitotic index has decreased from 16.82 % of control to 16.54 % in 100 ppm, 14.80 % in 250 ppm, 14.28 % in 500 ppm, 13.44 % in 750 ppm and 13.36 % in 1000 ppm concentrations. The data indicates that the fungicide is potentially mitodepressive. Similar mitodepressive nature of Endosulphan and Zineb were studied by Aktac *et.al.* (1994) and Kozera and Klein (1976) respectively.

At interphase, only one type of nuclear aberration *i.e.* binucleate cells was observed in all the concentrations except 1000 ppm concentration. The highest percentage of the binucleate cells was found in 100 ppm concentration. Binucleate cells are the result of delay or failure of cytokinesis as reported by Ene-Oblong and Amadi (1987). Binucleate cells were also observed by Pandita and Khoshoo (1985) during testing of mutagenicity of Thimet 10 - G in *A. cepa*. A number of pesticides such as bromacil (Asthon *et.al.*, 1969), carbaryl (Amer and Farah 1968), Dinoseb (Sawamura 1965), Hexachlorocyclohexane (Baquar and Khan 1971) and nitraline (Genter and Burk 1968) are known to induce binucleate and multinucleate conditions.

At metaphase, chromatid separation and polyploid cells were observed. The 750 ppm concentration of fungicide is capable of inducing maximum percentage of polyploid cells (17.14%). All the tested concentrations are able to cause chromatid separation but 100 ppm of aqueous solution of cycloheximide caused highest percentage of chromatid separation (21.87%). Polyploid cells were observed only in higher concentrations.

At anaphase, only bridges were observed in higher concentrations and it's percentage was increased with increasing concentration.

At telophase, bridge formation and laggards were observed. The laggards were observed only in 100 ppm concentration (13.04%). The telophasic bridges were frequently observed in all the concentrations except 1000 ppm. These bridges were also studied by Somashekhar *et. al.* (1982) when they studied the clastogenic effects of vitavaxe in *A. cepa* L.

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