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Monitoring of morphotoxic, cytotoxic and genotoxic potential of mancozeb using *Allium* assay

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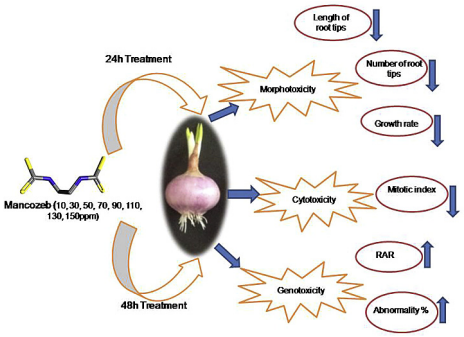
HIGHLIGHTS

- Genotoxicological assay using bulb of *Allium cepa* was carried out with Mancozeb.
- It is applied to fields leading to the accumulation of its breakdown products.
- *Allium cepa* root system is sensitive indicator of environmental pollution.
- Result indicated significant morphotoxic, cytotoxic and genotoxic effect.
- The toxic and mutagenic effects increased with time of treatment.

GRAPHICAL ABSTRACT



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ABSTRACT

The present experiment was designed to monitor the morphotoxic, cytotoxic and genotoxic potential of Mancozeb (fungicide) in non-target plants using bulbs of *Allium cepa*. Mancozeb is classified as a contact fungicide and is registered for use on a variety of crop plants. In the present monitoring, *Allium cepa* bulbs were exposed to different concentrations of mancozeb *viz.*, 10, 30, 50, 70, 90, 110, 130 and 150 ppm for 24 and 48 h. The potential morphotoxic and cytotoxic effects of mancozeb were examined by determining the average root number, average root length, mitotic index, relative abnormality rate (%) and frequency of abnormalities (%). A progressive significant concentration and time dependent inhibition of the average root number, average root length indicated the morphotoxic nature. The cytotoxic effect was significantly increased for 48 h treatment as compared to 24 h treatment time, by reducing the mitotic index of meristematic cells. The results indicated an indirect genotoxic effect by inducing different types of chromosomal abnormalities, likely sticky, disoriented and fragmented chromosomes. Thus indicating that the investigated fungicide have genotoxic potential due to abnormal DNA condensation and chromosome coiling by spindle inactivation. The observations of cyto and genotoxic effects suggest that the fungicide mancozeb is clastogenic agent. Thus the different concentrations used in the field could be harmful for the end-receptors of food-chain and needs constant monitoring and management for the better development of crop plants.

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1. Introduction

Fungicides, a class of pesticide have long been used to control, prevent and remediate microbial growth. The use of fungicide is one of the most important aspects in agriculture for protection of seeds during storage as well as in field by preventing the growth of fungi that may produce toxins. Currently public concern about the impact of fungicides on humans, birds, fishes and beneficial microorganisms that are exposed directly through various ways and indirectly through diet has increased. This is because fungicides tend to be applied repeatedly over a specific period of the year, so arguably pose a greater environmental risk than other types of pesticides, such as insecticides, which tend to be applied more intermittently to eradicate pest outbreaks when detected (Yoon et al., 2013). In the present study the fungicide tested is mancozeb (MZ), a manganese ethylene bis-dithiocarbamate polymer coordinated with zinc ion (Fig. 1), used widely since 1967 to protect various field crops, fruits, vegetables and ornamental plants against fungal diseases.

Mancozeb is classified as a contact fungicide, which can increase the yield parameters of crop plants by reduction of diseases soon after its application. It is registered for use on a variety of vegetable, fruit, nuts and grain crops and is marketed by different trade names. It is also used for seed treatment of cotton, potatoes, corn, safflower, sorghum, peanuts, tomatoes, flax, and cereal grains. MZ belongs to a group of fungicide known as ethylene bis-dithiocarbomates which itself is not fungicidal but when exposed to water, break down to release ethylene bisiothiocyanate sulphide (EBIS) which in turn via the action of UV light is converted into ethylene bisisothiocyante (EBI) (Geissen et al., 2010). EBIS and EBI are thought to interfere with biochemical processes within the fungal cell cytoplasm and mitochondria resulting in inhibition of spore germination. Thus it acts by inhibiting enzyme activity in fungi by forming a complex with metal containing enzymes including those involved in production of adenosine triphosphate (Enyiukwu et al., 2016; Utlwang et al., 2016). It is reported to cause structural and functional changes in thyroid of rats and also affect level of glycogen, proteins and lipids in testis, liver and kidney (Axelstad et al., 2011; Ksheerasagar and Kaliwal, 2010). MZ induces genotoxicity and apoptosis in cultured human lymphocytes (Srivastava et al., 2012). A dose-dependent increase in spindle morphology alterations due to indolfil (MZ) treatment in *Lens culinaris* was reported by Roy et al. (2014).

Studies have been carried out to detect harmful effects of fungicides as well as their undesirable residues in water, food and environment as they may cause some serious health problems (Petit et al., 2008). Chromosomal abnormalities induced by some of these compounds were found to be linked with their capacity to induce mutations (Panday et al., 1994) and can therefore be regarded as consistent evidence for the evaluation of genotoxicity (Grant, 1982). Plant genotoxicity assays are relatively inexpensive, fast and give reliable results and chemicals which cause chromosomal alterations in plant cells also produces chromosomal abnormalities in cultured animal cells that are frequently identical (Grant, 1978). The plants, being direct recipients of agro toxics, become important material for genetic test and for environmental monitoring of cases affected by such products (Sharma and Paneerselvan, 1990). Several plant test systems are already in use and are found to be as sensitive and reliable as other short-term tests.

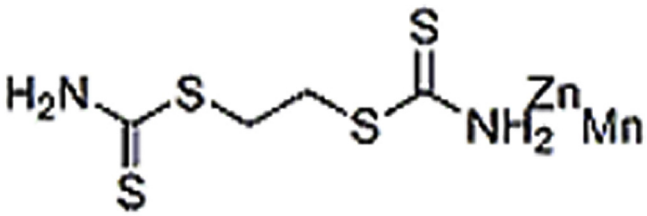

Fig. 1. The chemical structure of Mancozeb.

Allium cepa (onion) has been considered as the best-established test systems to indicate the presence of mutagenic chemicals due to sensitive dynamics of root growth, clear mitotic phase, stable chromosome number with kinetic characteristics of proliferation thus suitable for cytogenotoxic study (Barberio et al., 2011; Firbas and Amon, 2013; Leme and Marin-Morales, 2009; Pathiratne et al., 2015; Sharma and Vig, 2012). Different parameters of *A. cepa* such as root growth rate, mitotic index, chromosomal abnormalities etc. can be used to estimate the cytotoxicity and mutagenicity of environmental pollutants *viz.,* fungicide in agricultural fields. When applied to soil, these chemicals retain their effectiveness for a considerable period of time and potentially pose a risk to the long-term fertility of the soil (Komarek et al., 2010; Wightwick et al., 2008).

The objective of this study was to monitor the morphotoxic and cytogenotoxic potency of commercial formulation of MZ by *Allium cepa* test system. It was done by recording and calculating the average number and length of root tips (morphotoxic effect) and by calculating mitotic index and frequencies of abnormalities in root tip cells of *A. cepa* L. with a view to detect their mutagenic potential (cytogenotoxic effect).

2. Materials and methods

2.1. Test chemicals

Mancozeb (75% W·P) was purchased from a local agricultural store in Lucknow, India. The other chemicals used in the present study were of analytical grade.

2.2. Test organism and fungicide application

Healthy and equal sized bulbs of common onion (*Allium cepa* L. 2n = 16), procured from local market of Lucknow. For experiment, the loose outer scales of bulbs and old roots were removed with the help of sharp and pointed forceps so as to expose the root primordia. Different concentrations of MZ *viz.,* 10, 30, 50, 70, 90, 110, 130 and 150 ppm were prepared by dissolving calculated amount in distilled water and a control of distilled water was maintained. A set of three bulbs were grown in three separate test-tubes for each concentration of MZ for two durations- 24 h and 48 h.

2.3. Morphological and cytological investigation

After treatment, the bulbs were washed thoroughly under running tap water. On third day, number and length of root tips were recorded for morphological analysis. The root tips from each bulb were plucked and fixed in fixative, Carnoy's fluid (1:3 glacial acetic acid: ethanol) for 24 h and then preserved in 70% ethanol for cytological analysis. Hydrolysis, squashing, staining of cells and preparation of slides was done according to the method outlined by Sharma and Sharma (1980). The slides were mounted and observed under the light microscope. Photographs of different mitotic stages and chromosome abnormalities observed were taken using digital camera. Counts of different mitotic stages were also recorded. Following formulae were used to calculate percentage mitotic index (MI), relative abnormality rate (RAR) and abnormality percentage.

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