

Non-Targeted Cytotoxic and Genotoxic Impacts of Agricultural Pesticides in Plants: A Comprehensive Review with few Case-studies

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Keywords: Agrochemicals, Pesticides, Cytotoxicity, Genotoxicity, Chromosomal aberrations, Mitotic index.

Abstract:

For decades, pesticides have been used religiously in crop fields not only to control pests but also as a hangover habit in India, despite knowing their prolonged and adverse environmental and biological consequences. In every season, a significant amount of pesticides, instead of reaching the target organisms, end up in soil and water bodies. From there, these pesticides accumulate inside the non-target species of plants, leading to several cytotoxic, genotoxic and adverse metabolic and physiological effects in plant systems, like cellular, chromosomal, and DNA-level alterations. During the last decade, pesticide bioassay studies using *Allium cepa*, *Vicia faba*, *Pisum sativum*, *Helianthus annuus* etc revealed that insecticides, fungicides, and herbicides induce oxidative stress, disrupt mitotic activity, impose a variety of chromosomal abnormalities, such as anaphase bridges, laggard chromosomes, sticky chromosomes, chromosome fragments, and metaphase arrest. These cytogenetic effects are often concentration- and exposure-dependent and are closely associated with reactive oxygen species (ROS) generation and disruption of cellular homeostasis. Moreover, due to increasing global use, these pesticides can be accumulated in the plants and other animals and enter into the food chain, alter the soil microflora therefore disturb the stability of ecosystem and human health.

Introduction

Several chemicals are widely used in agriculture not only to improve crop productivity but also to prevent crops from unwanted pests, such as insects, microbes that can cause disease, and weeds that suck nutrients from the soil. Unfortunately, the absolute dependence on those chemicals for decades is now showing adverse effects on the environment, ecological imbalance, and human health. Evident from several leading research studies, the agrochemical residues contaminate surface runoffs, water bodies, soil, and eventually groundwater and come up with severe negative impact on surrounding biota and ecological balance (Sharma et al., 2019; Shahid et al., 2019a). According to Sharma et al. (2019) and Shahid Zaidi, Ehtram & Khan (2019b),

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approximately 3 million Tons of chemical pesticides are used worldwide each year, of which a substantial proportion ends up in the environment, poisoning non-target organisms.

Despite of the threats posed by the pesticides, environment is constantly trying to self-cure itself by several means, like bacterial degradation, photodegradation etc and reduces the ecotoxic effects to some extent (Salazar Mercado & Correa, 2024), but the globally increasing use of numerous agrochemicals magnifies their footprint in the environment manifold and enhances the potential risks in cumulative way on the ecosystem as a whole, from lower tropic level to the higher ones. Pesticides penetrate the ecosystem through soil, damaging its microflora and thereby reducing its fertility and water- and nutrient-holding capacity. Then they enter the primary producers (i.e., plants), after damaging their growth, development and overall productivity, and are accumulated at different levels of consumers, including humans. (Shahid & Khan, 2019a & 2019b; Rani et al., 2021; Hatamleh et al., 2022).

The entry of Pesticides into the plant system

A significant amount of agrochemicals applied to crops leach into the soil almost instantly. The rest is washed away by rain into the nearby soil. All plants, including microbes growing in that soil, become exposed to those chemical compounds. When absorbed by plant roots, these chemicals induce high oxidative stress by producing large amounts of reactive oxygen species (ROS). This ROS induces destabilization of the cell membrane, leading to ion leakage across the membrane and, consequently, impaired metabolic activity and cell death (Rani et al., 2021). These physio-chemical changes often alter another cellular function i.e., cell division. It is evident that when exposed to pesticides, several plants (perhaps all) show visible, disruption and reduction in mitotic activity with severe anomalies in metaphase and anaphase chromosomes, which indicate cytotoxic as well as genotoxic potentials of those pesticides directly or indirectly on the plants like *Vicia faba*, *Allium cepa* etc (Amer et al., 1999; Sheikh et al., 2020). These mutagenic properties of those agrochemicals are very much alarming for the long-term sustenance of ecology of any area of concern.

Genotoxicity and Cytotoxicity

Genotoxicity can be defined as the act of damage to DNA (genetic material) of any organism by any externally introduced agent in any form, causing mutation, chromosomal aberration, cancer, etc. (Hatamleh et al., 2022; Rani et al., 2021). Genotoxic materials may damage or mutate the DNA directly or interrupt the proof-reading activity and/or repair mechanism during the DNA replication process. However, not all genotoxic agents cause permanent damage to genetic material. (Amer et al., 1999; Sheikh et al., 2020).

Cytotoxicity, on the other hand, is a rather manifestation of genotoxicity and/or other external influences. Any external agent that alters, disrupts or inhibits cell division and thereafter kills the cell is cytotoxic agent. The symptoms of cytotoxicity in plants include stunted growth,

wilting and necrosis and apoptosis. Some cytotoxic agents sometimes damage DNA, but most of them primarily destroy membrane integrity, increase oxidative stress, inhibit several metabolic enzymes, and induce programmed cell death cascade. To quantify different aspects of cytotoxicity, few assays were formulated such as i) Lactate Dehydrogenase assay which measures degree of membrane damage or ii) 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide assay which quantifies metabolic activity of a cell (Hatamleh et al., 2022; Rani et al., 2021; Sheikh et al., 2020).

Cytotoxicity on plant system

Pesticide	Effects
Benomyl	decreased mitotic activity, anaphase bridges, sticky and lagging chromosomes in <i>Vicia faba</i> and <i>Pisum sativum</i> (Amer et al., 1999).
Chlorpyrifos, Alpha-thrin, Efekto virikop and Springbok	Metaphase and anaphase chromosomal anomalies, reduced mitotic activity in <i>Allium cepa</i> (Asita and Makhalemele, 2008).
Cypermethrin	decreased mitotic index and chromosomal aberrations in <i>Helianthus annuus</i> (Inceer et al., 2009); and <i>Allium sativum</i> (Saxena et al., 2005).
Metolcarb	High mutagenic activity on <i>Salmonella</i> (Liman et al., 2010)
Endosulfan, Dieldrin, and Aldrin	Metaphase disruption, C-mitosis, Sticky and laggard chromosomes, precocious movement with late and uneven separation of chromatids in anaphase in <i>Vicia faba</i> (Pandey, 2008)

Pandey in 2008 found that pesticides like Endosulfan, Dieldrin, and Aldrin reduce the mitotic activity at 500 ppm or higher concentrations while at lower concentrations somehow promote the same. Sheikh et al. (2020) showed that every part of a crop plant can absorb malathion and cypermethrin upon contact, causing significant harm to the plant and the environment.

Case Studies on two very common insecticides widely used in India

Monocrotophos:

Monocrotophos, commonly known as Azodrin or Nuvacron, is classified under organophosphates and acts as an insecticide. By nature, the chemical is Dimethyl (E)-1-methyl-2-(methylcarbamoyl)vinyl phosphate, having the chemical formula C₇H₁₄NO₅P. In southern

and south-eastern Asia, including India, the Subcontinent of Africa, Monocrotophos is widely used during Cotton, tobacco, peanut, and other vegetable farming (Kaur & Goyal, 2019).

Mancozeb:

Mancozeb is a widely used Broad-spectrum systemic fungicide. It is a coordination polymer complex of Manganese ethylene-1,2-bis(dithiocarbamate) with zinc salt, having empirical formula $(C_4H_6N_2S_4)Mn \cdot Zn$. Mancozeb has been used over the subcontinents of Africa, Asia, Europe, North and South America. Countries like India, Brazil, China and several parts of Africa still use this chemical in agriculture. However, now-a-days in European Union and America it is banned for its harmful impact on environment (Dall'Agnol et. Al. 2021)

Material and Methods

Pesticides were purchased from the local market of Krishnanagar, Nadia, West Bengal. First, a stock solution of 1000 ppm was prepared, which was diluted to prepare three solutions - 100 ppm, 250 ppm and 500 ppm for treatment. The test system was an onion (*Allium cepa*), bought from the local market. The onions were kept in water till proper rooting. The rooted onions were treated with each pesticide concentration for 6 hours and 12 hours, then mitotic cell division in the root tip meristems was studied. After treatment, the root tips were cut out, washed with water and fixed for 1 hour to overnight in acetic acid and ethanol mixture (1:3). The materials were then kept for 5 to 15 minutes in 45% acetic acid (for storing, these were transferred to 70% ethanol and kept in the refrigerator). The materials were then transferred to a mixture of 2% acetic-orcein and (N) HCL (9:1) and then gently warmed for 2-5 seconds. The materials were kept in a staining mixture for 30 minutes to 1 hour. Each root tip (deep coloured) was placed in a drop of 45% acetic acid on a clean, dry slide, covered with a cover glass, and tapped with a matchstick to scatter the cells, and uniform pressure was applied to spread the material. Excess fluid was blotted out, sealed with paraffin and the slides were observed under microscope. Mitotic index, percentages of individual divisional stages as well as the aberrations and abnormalities in different stages were recorded from the treated and untreated (control, kept in distilled water) root tips.

Results

It was observed that both Monocrotophos and Mancozeb have severe cytotoxic potentials. When compared to the control, both the pesticides showed elevated metaphase and anaphase abnormalities and significantly reduced Mitotic index (Figure 1 & 2). However, Mancozeb showed rather higher metaphase arrest than Monocrotophos, which was evident from the occurrence of more metaphase stages (though abnormal) than anaphase. On the other hand, Monocrotophos-treated root tips showed comparatively more anaphases than Mancozeb, most of

which were abnormal. Both chemicals at higher concentrations exhibited greater cytotoxicity and for each concentration, longer exposure led to higher cytotoxicity (Table 1).

Table 1. The effect of different concentrations of Monocrotophos and Mancozeb for different exposure times on the mitotic index, frequencies of different divisional stages and abnormality in the stages.

Treatments	Conc. (ppm)	Time of exposure (Hr)	MI	Pro (%)	Meta (%)	Ana (%)	Telo (%)	Abnormality (%)
Control	-	-	27.3	64	18	12	6	0
MCP	100	6	9.3	45.8	24.6	29.6	0	6.7
		12	4.6	42.7	24.3	32.4	0.6	23.5
	250	6	5.3	50.5	21.7	27.8	0	33.3
		12	2.7	49.2	23.3	27.5	0	41.7
	500	6	1.3	61.14	16.5	22.3	0.06	68.5
		12	0.2	67.9	11.6	20.5	0	82.5
MZ	100	6	13.3	41.9	38.2	19.6	0.3	8.6
		12	9.2	44.1	36.6	19.3	0	31.5
	250	6	8.7	50.2	32.3	17.5	0	46.6
		12	4.5	54.88	28.5	16.6	0.02	52.5
	500	6	2.6	55.7	27.6	16.7	0	77.8
		12	1.5	59.2	26.6	14.2	0	88.5
Abbreviations:- MCP = Monocrotophos; MZ = Mancozeb; Conc. = Concentration; MI = Mitotic index; Pro = Prophase; Meta = Metaphase; Ana = Anaphase; Telo = Telophase.								

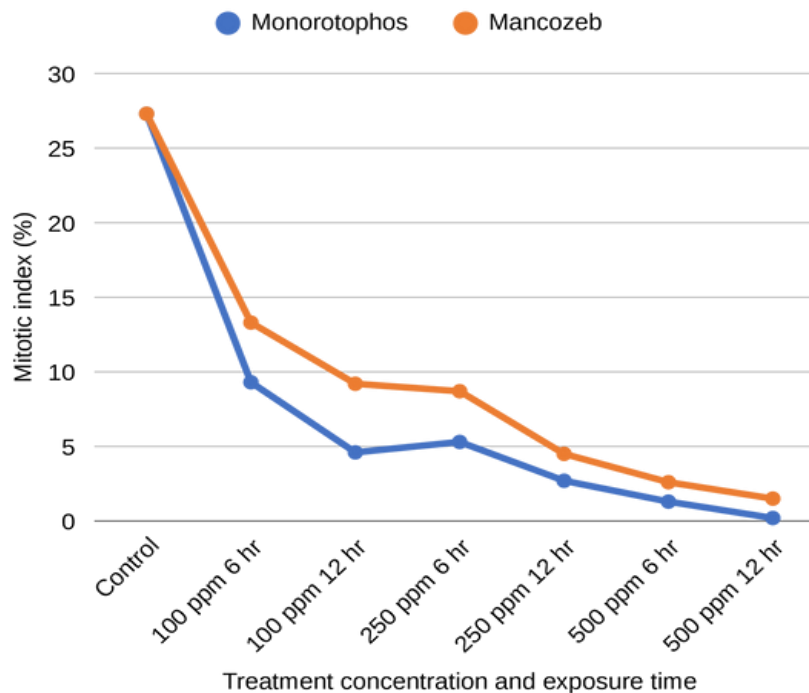


Figure 1. Comparison of mitotic indices in the root tip meristem treated with Monocrotophos and Mancozeb.

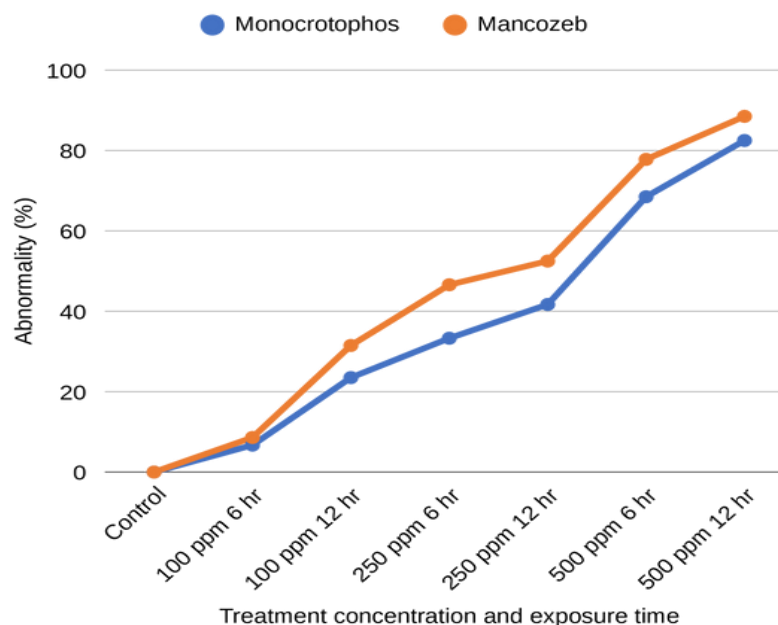


Figure 2. Comparison of the % of chromosomal abnormalities in the mitotic division of the root tip meristem treated with Monocrotophos and Mancozeb.

The abnormalities recorded during the studies were of different kinds (Figure 2), among which scattered metaphase, lagging chromosomes in anaphase, sticky chromosomes in anaphase, fragmented and multipolar chromosomal arrangements, etc., were very prominent (Figure 3, images 1 - 9).

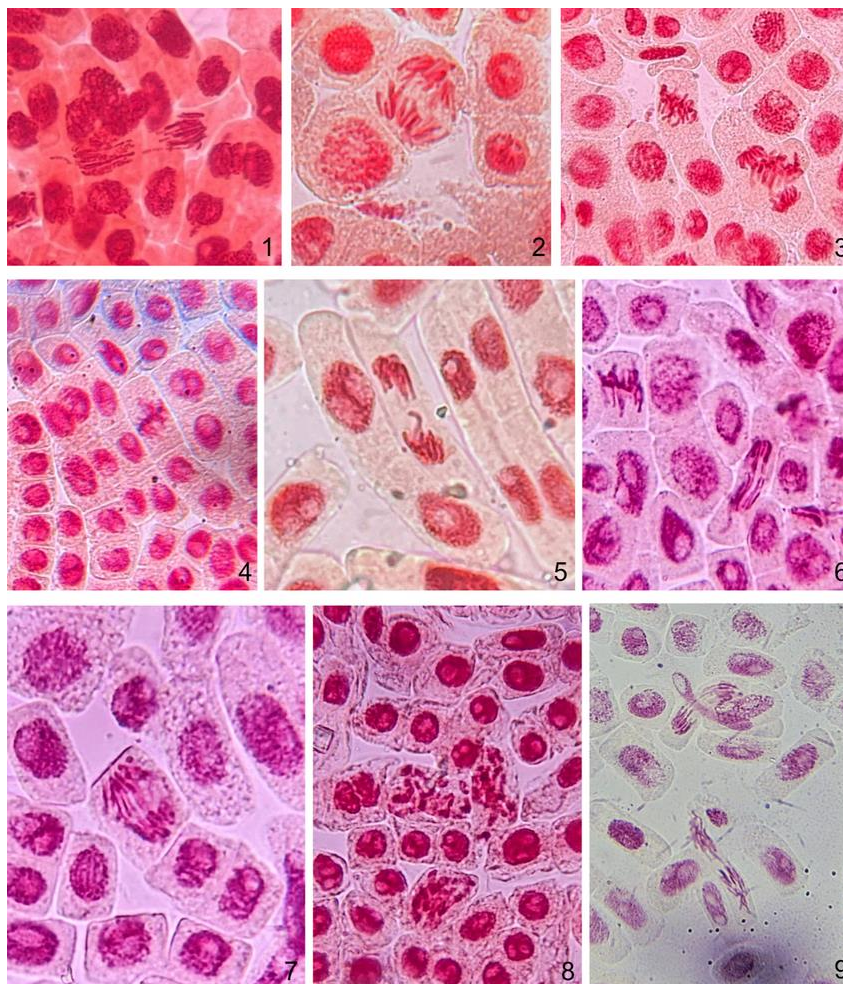


Figure 3. [Images 1–5]. Different concentrations and exposure of Monocrotophos-treated *Allium cepa* root tips. Image 1 shows lagging chromosome in anaphase when treated with 500 ppm for 6 hours; image 2: sticky chromosome in anaphase when treated with 250 ppm for 6 hours; image 3: scattered metaphase in 250 ppm for 12 hours; image 4: Scattered metaphase in 250 ppm for 6 hours; image 5: lagging chromosome in anaphase when treated with 250 ppm for 12 hours. Images 6 -9: Different concentrations and exposure of Mancozeb treated *Allium cepa* root tips. image 6: sticky chromosome and fragmented chromosome in anaphase when treated with 250 ppm for 6 hours; image 7: anaphase bridge in 250 ppm for 12 hours; image 8: scattered metaphase in 500 ppm for 6 hours; image 9: multipolar anaphase in 500 ppm for 12 hours.

Concluding Remarks

In the modern perspective and the huge pressure of consumerism, it is an unavoidable practice to use different agrochemicals without properly knowing their long-term side effect on the environment, the ecosystem, and finally human health. The apparent promise of protection of crops from pests leads to a potential depletion in the productivity of all crops. Several researches including the current case studies clearly stated that those pesticides have a tremendous cytotoxic and genotoxic effect not only on the pests, but also on the crop itself, if not considered the human as well as animal health hazards. Most developed countries have banned the use of several agrochemicals, but developing countries like India, Africa, etc., still use them. Therefore, there is a dire need to develop somewhat safer agrochemicals or stop using them.

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HOW TO CITE

Alokemoy Basu (2025). Non-Targeted Cytotoxic and Genotoxic Impacts of Agricultural Pesticides in Plants: A Comprehensive Review with few Case-studies. © International Academic Publishing House (IAPH), Dr. Nithar Ranjan Madhu, Dr. Prosun Bhattacharya, Dr. Sarka Nedomova, Dr. Tanmay Sanyal and Mr. Rohit Sahu (eds.), *A Basic Overview of Environment and Sustainable Development*, Vol. 4, pp. 50-59. ISBN: 978-81-989555-0-0
doi: <https://doi.org/10.52756/boesd.2025.e04.002>

