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Cytotoxicity of Ambucrop Fungicide on Root Tip Cells of *Allium cepa* (2n=16)

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ABSTRACT

Fungicides are agrochemicals that are specifically designed to minimize agronomic loss and increases economic profit. But such chemicals degrade at a slow rate and may become persistent in the soil causing natural contamination. These fungicides also can exert genotoxic, cytotoxic and phytotoxic effect on target plants. Such detrimental effect may extend to other non targeted plants and to other biotic species causing ecological imbalance. Different concentrations of Ambucrop fungicide (25ppm, 50ppm, 75 ppm and 100 ppm) exerted negative impact on the Mitotic index of root tip cells of *Allium cepa* (2n=16) with increasing the abnormality index as compared to the control. The cytotoxic effect increased not only with increasing the concentration of Ambucrop fungicide, but also on the duration of the treatment.

***Key words**: Cytotoxic, ppm, Mitotic index (MI), Abnormality index (ABI), Allium cepa (2n=16), aberrations*

Introduction

The first recorded use of pesticides was about 4500 years ago by Sumerians who used sulphur compounds to control insects and mites. Inorganic substances, like sodium chlorate, sulphuric acid used in pest control in the early 19th century which were overtaken by organics such as nitro phenols, chlorophenols, creosote, naphthalene and petroleum oils were to control fungal and insect pest. In 1990, Glyphosate was introduced which later became the highest selling herbicide followed by sulfonylurea and imidazolinone. One negative site of these pesticides was the high rate of application which can cause accumulation and toxicity. Synthetic pesticides were introduced in the past to increase agricultural crop productivity to meet up with the demand of rapidly growing global population and has played an effective role to withstand biotic and abiotic stress (Kumar and Verma, 2018). Day by day the pathogens are becoming resistant to these synthetic fungicides, which further increases the demand for a new formulation of fungicides to protect the crops (Parween *et al.*, 2016). A very small percentage (>0.1%) of total applied fungicide concentration reach the target site while a major portion of the applied fungicide accumulate on off-target sites causing damage to non target crops (Rio *et al.*, 2012). Over dose or repeated pesticide application shows detrimental effects on the crops by damaging the important biomolecules and leading to production of reactive oxygen species (ROS) (Anitha and Savitha, 2015). Above the permissible dose, fungicides affect cell development, promote chromosomal aberrations, failure of photosynthesis along with retardation of biosynthesis and molecular responses at different stages of plant life. The unacceptable fact is that over doses or repeated doses of

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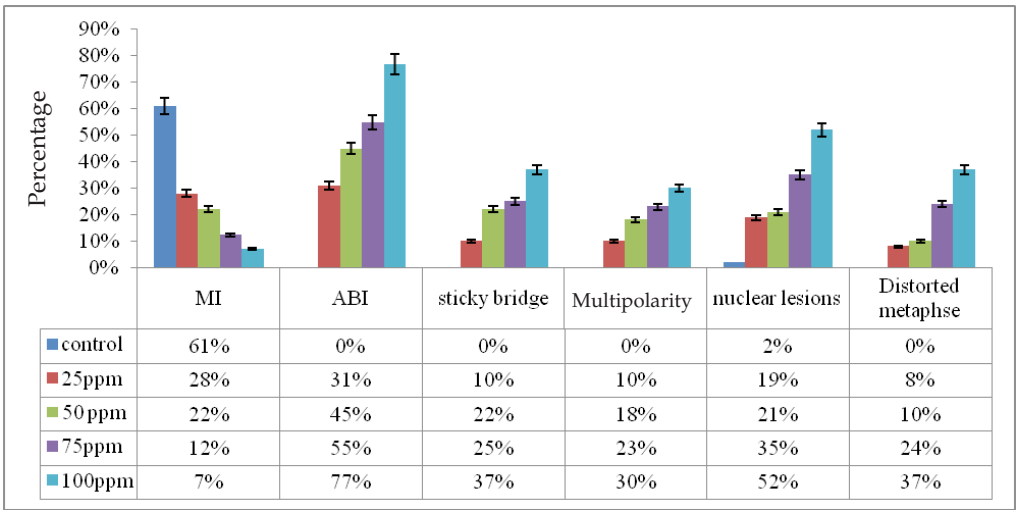
pesticide application shows detrimental effects on the host plant. Increased use of fungicides and pesticides leads to cytotoxic effects, genotoxic effects and chromosomal aberrations along with DNA damage and increased level of mutation (Anitha and Savitha, 2013). In the mango orchards of Malda district of West Bengal, different fungicides are applied time to time to increase the yield of mango by minimizing the loss caused due fungal pathogen attack. The most commonly used fungicides includes the group of synthetic pyrethroids, mancozeb, organophosphate and carbendazim. Mancozeb group of fungicide is reported to induce spindle inactivation resulting in chromosome coiling and also impart stickiness to the chromosome resulting in improper separation (Fatma *et al*., 2018). As per UNEP (United Nations Environment Programme), *Allium cepa* (2n=16) is one of the most established system to study mitotic abnormalities due to its large chromosome size and low chromosome number. The entire process is cheap and low time consuming which makes it an effective system to test the toxicity of different fungicides and pesticides (Rank, 2003). Ambucrop is a mixture of mancozeb and carbendazim fungicide that remains on the plant surface and effectively controls blast, leaf spot and other related fungal diseases. The current study demonstrates the cytotoxic effect of commonly used fungicide of mango plants, Ambucrop, on the root tips of test plant *Allium cepa* (2n=16).

Materials and Methods

Equal sized bulbs of *Allium cepa* (2n=16) were procured from local markets and were surface sterilized with 1% sodium hypochlorite solution. The bulbs were then placed on water filled small glass containers placed at 25±2°C for 48 hours allowing the roots to grow. Equal sized roots *Allium cepa* (about 1 cm) were then treated with fungicide solution Ambucrop for four different concentrations viz (25 ppm, 50 ppm, 75 ppm & 100 mg/l) for 2 hours and 4 hours duration. Root tips of *Allium cepa* (2n=16) maintained in distilled water served as control. After 2 and 4 hours of treatment, 5-6 root tips were excised and fixed in 1:3 acetic ethanol for 24 hours at 12-14 °C. After that, the root tips were squashed using 1(N) HCl with mild heating and stained using 2% acetic orcein for 40-50 minutes (Ray *et al*., 2013). Finally the root tips were squashed using 45% acetic acid on grease free slides and observed under light microscope. Mitotic index was calculated following the formula as Mitotic Index (MI) $=\frac{TDC\times 100}{TC}$, whereas Abnormality index (ABI) were calculated as = $\frac{TC\times 100}{TDC}$. Similarly the different Abnormal phases were calculated as $\frac{TC\times 100}{TDC}$where TDC = Total dividing cells TC = Total dividing and non-dividing cells (Barman *et al*., 2020).

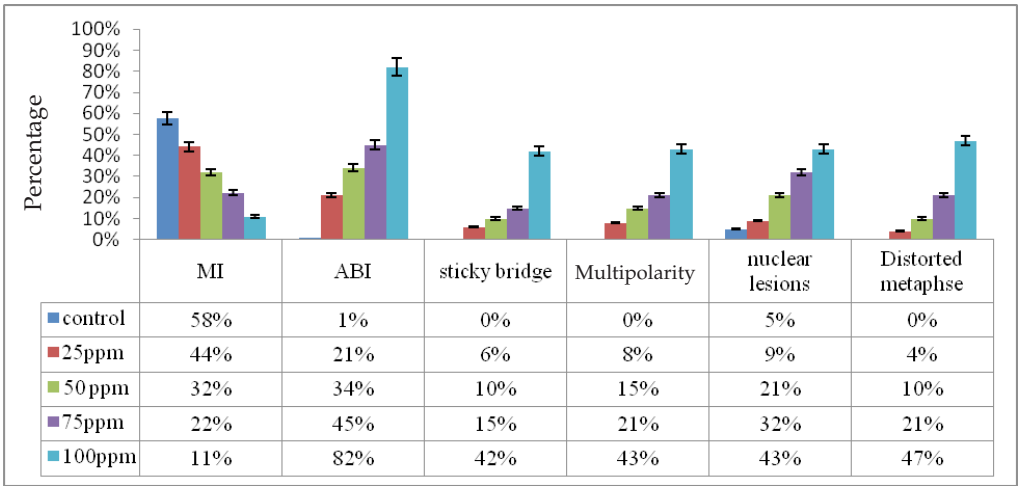
Results and Discussion

Figure 1 and Figure 2 through bar graph shows the effect of different concentrations of Ambucrop fungicide on the mitotic activity of *Allium cepa* (2n=16) root tip meristematic cells. The root tip cells treated with distilled water for 2 hours and 4 hours showed the highest mitotic index with no abnormal stages of divisions as compared to other treatments. 25 ppm of Ambucrop solution induced the lowest abnormality index as compared to the other concentrations of Ambucrop fungicides in *Allium cepa* (2n=16) treated for both 2 hours and 4 hours. On increasing the concentration of Ambucrop, the abnormality index also increase proportionately in *Allium cepa* (2n=16) root tips with highest at 100 ppm concentration. Similarly time duration also showed direct correlation with concentration of treatment solution of Ambucrop on the root meristem cells of *Allium cepa* (2n=16). It has been observed that with the Mitotic index dropped rapidly with increase in the concentration of the treatment solutions and also with the time duration. Different types of chromosomal aberrations (sticky bridge, anaphase bridge, nuclear lesions, multi polarity) were also found to be highest in the root tip meristematic cells of *Allium cepa* (2n=16) treated for 4 hours. Among the divisional abnormalities observed, multi polarity and sticky bridge were found in greater numbers in the all the treatments. Stickiness of chromosomes may be due to inhibition in spindle formation and may also result in improper folding of the chromatids resulting in their stickiness (Bianchi *et al*., 2016). Multipolar anaphase cells resulted due to depolymerisation of spindle fibers which and subsequent shifting of poles were noticed. The sticky nature of the chromatids during anaphasic separation might also have occured due to denaturation of the nucleic acid resulting in entanglement of the chromatin fibres (Evandri *et al*., 2000). The chromatin materials fail to



	MI	ABI	sticky bridge	Multipolarity	nuclear lesions	Distorted metaphse
control	61%	0%	0%	0%	2%	0%
25ppm	28%	31%	10%	10%	19%	8%
50ppm	22%	45%	22%	18%	21%	10%
75ppm	12%	55%	25%	23%	35%	24%
100ppm	7%	77%	37%	30%	52%	37%

Fig. 1. Bar Graph showing Mitotic Index (MI), Abnormality Index (ABI), Sticky Bridge, Multi polarity, Nuclear Lesions and distorted metaphase in *A.cepa* root tips (2n=16) treated with different concentrations of Ambucrop Fungicide for 2 hours.



	MI	ABI	sticky bridge	Multipolarity	nuclear lesions	Distorted metaphse
control	58%	1%	0%	0%	5%	0%
25ppm	44%	21%	6%	8%	9%	4%
50ppm	32%	34%	10%	15%	21%	10%
75ppm	22%	45%	15%	21%	32%	21%
100ppm	11%	82%	42%	43%	43%	47%

Fig. 2. Bar Graph showing Mitotic Index (MI), Abnormality Index (ABI), Sticky Bridge, Multi polarity, Nuclear Lesions and distorted metaphase in *A.cepa* root tips (2n=16) treated with different concentrations of Ambucrop Fungicide for 4 hours.

condense properly during divisional stages which create a sticky nature of the chromosomes (Ford and Correl, 1992). Unequal breakage and reunion of chromatids results in the formation of Anaphase Bridge which is also a result of the sticky nature of the chromatids that prevents its separation (Kumar and Tripathy, 2003). Irregular accumulation of spindle microtubules resulted in disorientation of spindle microtubules causing distorted metaphase in the treated root tip cells of *Allium cepa* (2n=16) (Turkoglu, 2007). All the abnormal cell types were given in Figure 3 (A-I).

Conclusion

Applications of different types of systemic fungicides are quite common in the mango orchards of Malda District. Among them, Ambucrop is quite commonly used to make the inflorescence and other plant parts of mango free from fungal attack and to increase the yield. Most of the mango orchards in Malda district are cohabitated by different agricultural lands which yields different economically important vegetables in different seasons. It is possible that excess of fungicides may run off into other

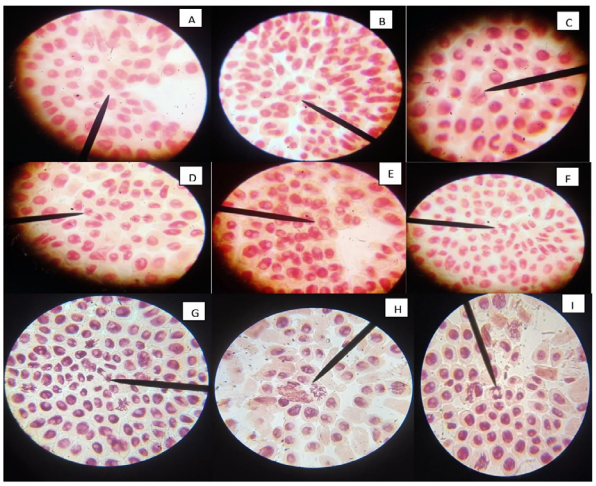


Fig. 3. A& C-Distorted metaphase, B & F-Sticky bridge, D &H Multi polarity, E- Nuclear Lesions, G &I – Anaphase Bridge.

crops and can induce cytotoxic effects as has been experimentally observed on root tip meristems of *Allium cepa* (2n=16). The most common abnormality induced by the treatments was stickiness in the chromosome which implies irreversible damage to the cells leading to programmed cell death (El-Ghamery *et al*., 2000). So it may be concluded that unlimited application of Ambucrop fungicide may lead to hazardous effect in other vegetable crops and may also enter the food chain to impose more detrimental effects.

Conflict of Interest: The authors express no conflict of interest.

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