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Genotoxic effect of hexaconazole on root meristem cells of *triticum aestivum* L.

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ABSTRACT

In vivo cytogenetic assay in *Triticum aestivum* L. root meristem cells has been carried out to study the effect of triazole fungicide - Hexaconazole on mitotic division, chromosomal aberration and induction of micronuclei. Pre-soaked (12 h) seeds were treated with different concentration of Hexaconazole viz. 6.25, 12.5, 25 & 50 ppm for 6, 12 & 24 h. The treated samples exhibited significant ($P<0.05$) increases in the frequency of chromosomal aberrations and decrease in mitotic index. These abnormalities were observed in a dose and duration dependent manner. The present study reveals that the Hexaconazole is potential toxic to the plants.

KEYWORDS

Hexaconazole;
Genotoxicity;
Triticum aestivum L.;
Chromosomal aberration;
Micronuclei.

INTRODUCTION

Pesticides are among the most widely used chemicals throughout the world. Many of these compounds, because of their environmental persistence, are present in the environment for many years to come, and as a result they are responsible for several adverse effects on human health other than acute intoxications. Although pesticides are indispensable to the modern agricultural practice, the biological use of these pesticides over the years have resulted in problems caused by their interactions with the biological systems in the environment and have deleterious effects on living organisms. Many reports have shown that some of the pesticides are mutagens[1, 2] causing the development of several types of cancer[3]. They may also lead to endocrine disruptions and other developmental deficit[4-6] as well as to chronic effects on human health[2]. Therefore, it is important to evaluate the potential genotoxic risk arising from occupational exposure to these substances.

Hexaconazole is a systemic fungicide which is being used widely for controlling the fungal pathogens on a variety of crops[7-9]. Though the toxicity of hexaconazole studied in various organisms, there is paucity information on higher plants. Hence, the present study was undertaken to assess the genotoxic effect of a commercial triazole fungicide – Hexaconazole by the use of short-term test mitotic indices, chromosomal aberrations and micronuclei in *Triticum aestivum* L. (Wheat).

MATERIALS AND METHODS

Preparation of test solution

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Hexaconazole 5% EC (Udaan – a systemic fungicide, Bharat Insecticide Ltd) was freshly prepared by diluting it with distilled water in four different concentrations viz, 6.25 ppm, 12.5 ppm, 25 ppm, 50 ppm.

Experimental design

Clean and healthy seeds were presoaked in tap water for 12 hr and germinated in Petri dish containing tap water. Then the germinated root tips were treated with above concentrations of Hexaconazole for 6, 12, & 24 hr. Control was treated with distilled water. For each experiment a minimum of 50 seeds were used. During the experimentation, the seeds were maintained at 25 ± 1ºC in a BOD incubator. After completion of the exposure, 10 roots from 10 seeds were excised and immediately fixed in freshly prepared acetic acid : ethanol (1:3) for cytogenetic analysis.

Cytogenetic assay

Cytogenetic assay was performed from slides prepared by haematoxylin squash technique[10]. A minimum of 5000 cells from 10 root tips were scored for determining the frequency of mitotic index (MI), chromosomal aberrations (CA), such as metaphase and anaphasic abnormalities and interphase with micronuclei (MN). For the analysis of MN, the criteria described by Majer *et al.*,[11] were followed.

Statistical analysis

All the results values are expressed as mean ± SE. Comparisons between the control and treated groups were evaluated by oneway - ANOVA using SPSS software package. Multiple comparisons were performed by Tukey's HSD test. $P<0.05$ was considered as the level of significance.

RESULTS

Effect of Hexaconazole on mitotic division

The frequency of mitotic index observed in root meristem cells of *Triticum aestivum L*. following treatments with Hexaconazole at four doses of different durations along with correspond control value and are presented in Figure 1. The treated cells exhibited a gradual decrease in mitotic index, during all doses and durations. These reductions in the frequency of cell division were statistically significant ($P<0.05$) when compared to control. This clearly indicates the mitotoxicity of Hexaconazole.

Effect of Hexaconazole on chromosomal aberration

Observation on the frequency of chromosomal aberrations and mitotic anomalies treated with Hexaconazole at various dose and durations are presented in Figure 2. It was observed that the Hexaconazole treated cells showed increase in the frequency of chromosomal aberration during all doses and durations of exposure. These increase in the frequencies of chromosomal aberration was statistically significant ($P<0.05$) when compared to control. Distinct dose dependent chromosomal aberrations were also observed. The treated cells ex-

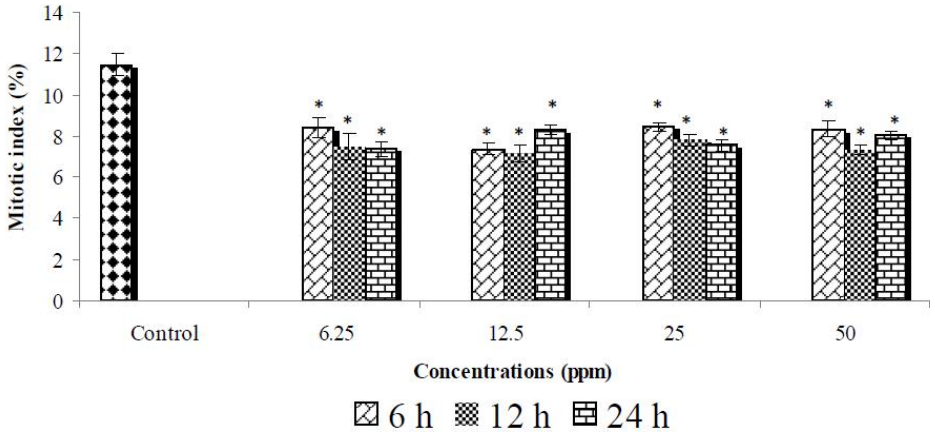

Figure 1 : The frequencies of mitotic index observed in the root tipcells of *Triticum aestivum* L. treated with fungicide - Hexaconazole

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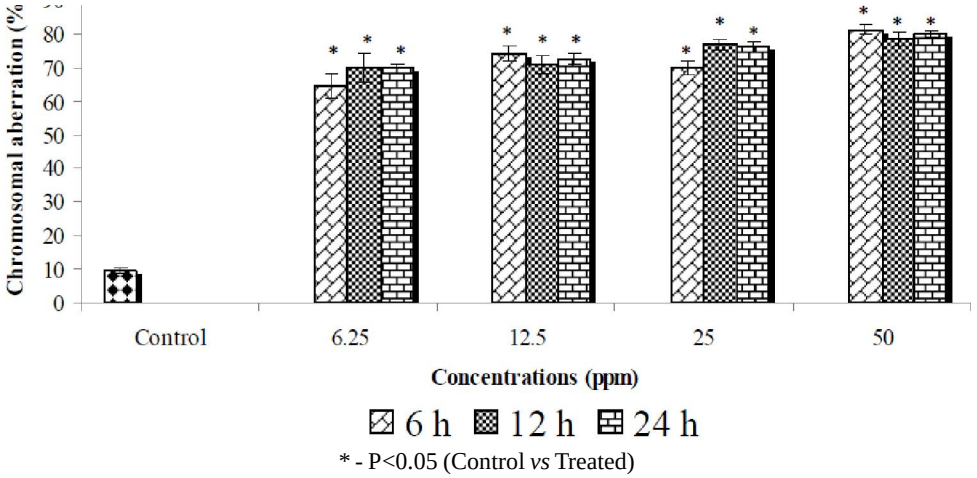

* - P<0.05 (Control *vs* Treated)

Figure 2 : The frequencies of chromosomal aberration observed in the root tip cells of *Triticum aestivum* L. treated with fungicide - Hexaconazole

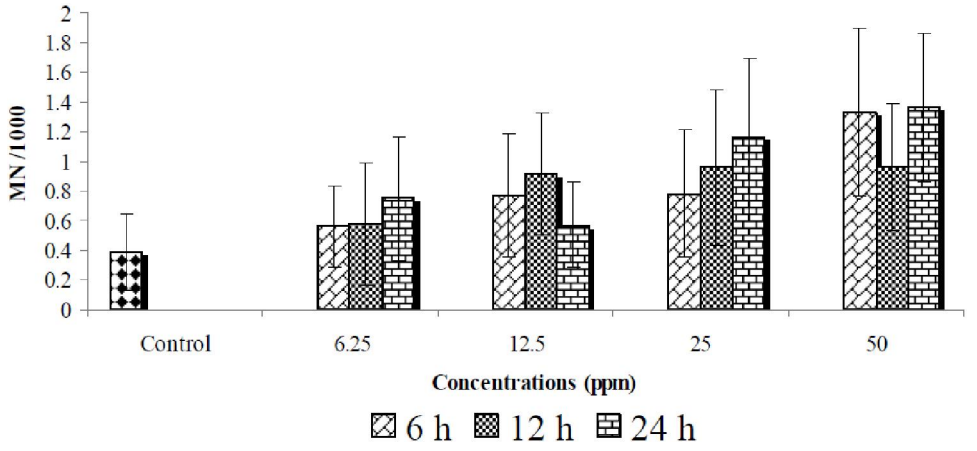

Figure 3 : The frequencies of micronuclei observed in the root tip cells of *Triticum aestivum* L. treated with fungicide -Hexaconazole

hibited wide spectrum of chromosomal aberration such as fragments, laggards, C – metaphase, sticky metaphase, disturbed metaphase, anaphasic bridge and disturbed anaphase.

Effect of Hexaconazole on induction of micronuclei

The results on the frequency of micronuclei are presented in Figure 3. It was observed that the treated cells showed slight increase in frequency of micronuclei during all concentration of exposure, however statistically this is insignificant when compared to the control.

DISCUSSION AND CONCLUSION

Different pesticides are used in agriculture and in house hold pest control. The extensive use of pesticides in modern agricultural practices has raised the question whether these chemicals induce any detectable cytological damage to the cells of living organisms or not. Though the use of these chemicals has become a necessity, their ingredients have induced acute toxic effects in different experimental systems[12]. These pesticides stay/persist in the environment for several years, cause toxicity for plants, animals and human beings as well. These pesticides cause serious effects on genetic materials of plants and induce genotoxicity.

In this work, we have investigated the capability of Hexaconazole to induce genotoxic effects. The research was focused on commercial formulations of this pesticide, bearing in mind that in this form they are applied in agriculture. Therefore, these commercial forms to which humans are exposed deserve

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a more thorough investigation.

The genotoxicity of Hexaconazole has been studied by several authors using different assays. In this study, we observed in the root meristem cells of *Triticum aestivum L*. treated with Hexaconazole at four concentrations exhibited significant decrease in mitotic index during all concentrations when compared to the control. This reveals the mitotoxicity of Hexaconazole. Similar observation also recorded by Yilmaz *et al*.,[13] in mouse bone marrow cells and human lymphocytes treated with Conan 5FL (Hexaconazole).

The cells treated with Hexaconazole showed significant increases in the frequency of chromosomal aberration and mitotic anomalies and these was found to be directly proportional to the concentration. This is akin to the earlier report. Yilmaz *et al*.,[13] found that Hexaconazole induced significant increases in the frequency of chromosomal aberrations in mouse bone marrow cells and human lymphocytes test systems. This fungicide caused structural and numerical abnormalities such as sister chromatid union, chromatid and chromosome breaks, fragments, dicentric and ring chromosomes, and polyploidy. Also significant increase was found in the induction sister chromatid exchanges at all treatments.

Induction of micronuclei was the result of chromosomal breaks and malfunctions of mitotic spindle. In this study it was observed that the induction of micronuclei is statistically insignificant ($P<0.05$). Some of the earlier reports on triazole compounds such Amitrole, a triazole herbicide did not increase mutation frequencies in test system involving two genera of bacteria, *Drosophila* and human lymphocyte cell cultures[14-16]. Bone marrow chromosomal aberrations assay indicated that hexaconazole is incapable of producing any structural or numerical aberrations and no effect on spindle formation during cell division[17]. The present study also confirms the genotoxic potential of Hexaconazole.

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