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Evaluation of Clastogenicity of 4, 6-Dinitro-*o*-cresol (DNOC) in *Allium* Root Tip Test

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ABSTRACT

4, 6-Dinitro-*o*-cresol (DNOC) is a pesticide, fungicide and insecticide, used in agriculture. In this research the genotoxic effect of DNOC on the root tips of *Allium cepa* L. was determined. The correlations between the effects of different concentrations, exposure times on the mitotic index (MI) and induction of chromosomal aberrations (CA) were also examined. Experiments were carried out in duplicate, using aqueous solutions of DNOC to concentrations of 250 and 500 ppm, at intervals of 0, 3, 6, 12, and 24 h. The results revealed an inhibition of the MI at the highest concentration and experiment times; this is an evidence of cytotoxicity of DNOC. Large number of c-mitosis indicated that DNOC acts as a strong spindle inhibitor, whereas, other CA types such as, breaks, bridges etc. were found in all tested concentration and times ($p < 0.001$) showing that is a potent clastogen.

Key Words: 4, 6-Dinitro-*o*-cresol (DNOC), mutagenicity, *Allium* test, clastogenicity.

INTRODUCTION

Dinitrophenolic compounds have a variety of uses for dye intermediates, wood preservatives, herbicides, insecticides, and sliming drugs (Gasiewicz 1991). 4, 6-Dinitro-*o*-cresol (DNOC), a dinitrophenolic compound, is one of the earliest synthetic organic pesticides which was developed in the 1890s for controlling tussock moth caterpillars, and used as fungicide, wood preservative and insecticide in more recent time (Hollingworth 2001). Such activity in a broad range of living organisms has enabled the development of dinitrophenol-derived herbicides and acaricides. A considerable number of dinitrophenolic pesticides, with a structure similar to DNOC, have been developed on the basis of their uncoupling effect on mitochondrial oxidative phosphorylation (Gasiewicz 1991; Palmeira et al 1994; Castilho et al 1997). However, their use as pesticides gradually declined in line with increasing knowledge of severe dinitrophenol toxicity in humans and animals.

DNOC is acutely toxic to humans. Workers may have been formerly exposed by inhalation or dermal contact during the manufacture, formulation, or application of the pesticide (Nehez et al 1981; Hollingworth 2001). Symptoms associated with DNOC toxicity are restlessness, a sensation of heat, flushed skin, sweating, thirst, deep and rapid respiration, tachycardia, severe increase in body temperature, and cyanosis leading to collapse, coma and death. Effects are enhanced at high environmental temperature (WHO, 2000). Nowadays, DNOC widely used in Turkey, while a few low-toxicity dinitrophenolic compounds are used as pesticides in other counties. In the year 2002, Turkish Ministry of Agriculture and Rural Affairs reported that 60646 kg DNOC was used against agricultural pests in Turkey.

With regard to DNOC toxicity in laboratory animals, acute lethal toxicity accompanied by depression, fever, dyspnea and cyanosis, induction of chromosomal aberrations and micronuclei in mouse bone marrow cells, and an increase of dominant lethal in mice have been reported (Nehez et al 1981; Gasiewicz 1991). DNOC was found positive in *Salmonella* test system (Nishimura et al 1982), *Drosophila* sex-linked recessive lethal test (Muller and Haberzetti 1980) and human lymphocytes chromosome aberration tests (Nehéz et al 1984) in vitro. Regarding these in vivo and in vitro studies, there is no report on genotoxic effect of DNOC in plant test systems.

Allium test has been recommended as a standard for cytogenetic assay in environmental monitoring due to the fact that data obtained with this plants show correlation with mammalian and non-mammalian test systems (Constantin and Owens 1982; Cauhan et al 1999). We aimed in the present study to determine the genotoxic effect of DNOC on *Allium cepa* root cells.

MATERIAL AND METHODS

Chemicals

4, 6-dinitro-*o*-cresol (DNOC, CAS no. 534-52-1, 90% pure) was obtained from Aldrich (USA). Ethyl methane Sulfonate (EMS, CAS no. 62-50-0) was obtained from Sigma Chemical Co. (St Louis, MO).

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Test Procedure

The assay was performed using 5 equal-sized young bulbs per sample of common *Allium cepa*. Onion bulbs were allowed to grow up to 2-2.5 cm root length. DNOC concentrations were 250 and 500 ppm and treatment times were 0, 3, 6, 12 and 24 h. After the treatment the roots were left in distilled water for 1 h. Then roots were fixed in acetic acid and ethanol (1:3) for 24 h and stored 70% ethanol at + 4ºC. The staining of the chromosomes was carried out with 1% aceto-orcein. After removing the root caps from well stained root tips, 1 mm of the meristematic or mitotic zones were immersed in a drop of 45% acetic acid on a clean slide and squashed under cover-slip by exerting thumb pressure on it.

One thousand cells per root from five bulbs (total of 5000 cells/sample) were scored for mitotic index (MI), as a measure of cellular division. All cells with alterations were counted. Anaphase-metaphase chromosomal aberrations (CA) consisted of bridges, pulverized and fragments, as DNA damage, and of laggards, multipolar anaphases, and c-mitosis, as mitotic spindle damage. The results were expressed as the percentage of anaphase-metaphase aberration. Negative control (distilled water) and a positive control (0.2% EMS) were also set up separately.

Statistical Analyses

Data were analyzed by one- and two-way analysis of variance (ANOVA/MANOVA) to test for overall differences among treatments related to the each parameter. Significance of differences amongst treatment means were assessed using a Tukey comparison test (HSD-test). All statistical analyses were based on a significance level of 0.05. The analyses were carried out with the commercial software programs SPSS 11.5.

RESULTS AND DISCUSSION

The results of this study were summarized in Table 1. MI is considered as a parameter that allows one to estimate the frequency of cellular division. The results of analysis of the effect of concentration and time of exposure to DNOC show decrease of the MI (Table 1) dependent on the concentration and time of treatment ($F = 26.789$, $p < 0{,}001$ and $F = 4.726$, $p < 0.005$, respectively). These results of MI are evidence of the toxic effect of DNOC on the exposed cells.

Table 1. Mitotic index and chromosomal aberrations after two concentrations and four treatment times of DNOC in *Allium* test and it statistical significance compare with negative control (ANOVA, at $p<0.05$).

Treatment	Time (h)	MI ± SD	C-Metaphases	Stickiness	Break	Total abnormalities
Control	0	0.34 ± 0.08	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	3	0.55 ± 0.11	0.03 ± 0.01	0.0 ± 0.0	0.01 ± 0.01	0.04 ± 0.01
	6	0.44 ± 0.17	0.03 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.08 ± 0.01
250 ppm	12	0.43 ± 0.09	0.19 ± 0.09***	0.06 ± 0.04	0.04 ± 0.01*	0.35 ± 0.10***
	24	0.49 ± 0.08	0.21 ± 0.04***	0.08 ± 0.06	0.05 ± 0.03**	0.56 ± 0.07***
	3	0.43 ± 0.06	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.08 ± 0.02
	6	0.34 ± 0.19	0.13 ± 0.03***	0.09 ± 0.03	0.02 ± 0.03	0.32 ± 0.08***
500 ppm	12	0.27 ± 0.09	0.19 ± 0.06***	0.21 ± 0.11***	0.02 ± 0.02	0.51 ± 0.04***
	24	0.14 ± 0.03	0.07 ± 0.09	0.20 ± 0.07***	0.0 ± 0.0	0.34 ± 0.07***

*, $p< 0.05$; **, $p< 0.01$; ***, $p< 0.001$

MI, Mitotic index; SD, Standard deviation.

The results of this investigation show a clastogenic effect which can be understood from the induction of CA at the concentrations used. It becomes significant ($p < 0.001$) beginning with 12 h and at 250 ppm of exposure (Figure 1). The two way ANOVA test analysis for related samples determined a significant dependence on concentration and the time of exposure for the percentage of CA ($F = 31.557$, $p < 0.001$). Breaks were the most commonly induced type of damage ($F = 7.415$, $p < 0.01$), suggesting a clastogenic

effect, besides of other aberrations types; gaps, bridges and pulverizations. In Figure 2, for both concentrations and exposure times, the induction of stickiness increases ($F = 14.771$, $p < 0.01$) after 6 hr of exposure. This phenomenon has been reported as indicative of high toxicity (Marcano and Del Campo 1995; Marcano et al 1998). Large number of c-mitotic anaphase indicates that DNOC acts as potent spindle inhibitor due to which all anaphase chromosomes lay on the metaphase plate instead of moving towards their respective poles ($F = 13{,}127$, $p < 0.05$; Figure 3). These anomalies such as c-mitotic effects and stickiness described previously and are considered as an inductor of aneuploids and poliploids (Fiskesjo 1985).

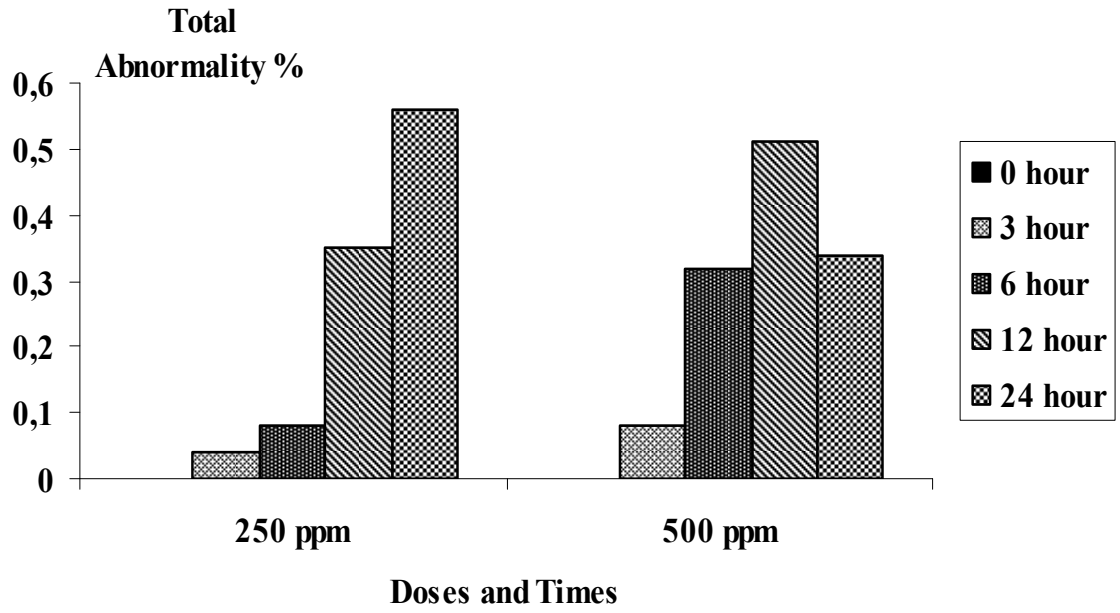

Figure 1. Induction of total chromosome aberrations with DNOC in the *Allium cepa* root tip cells

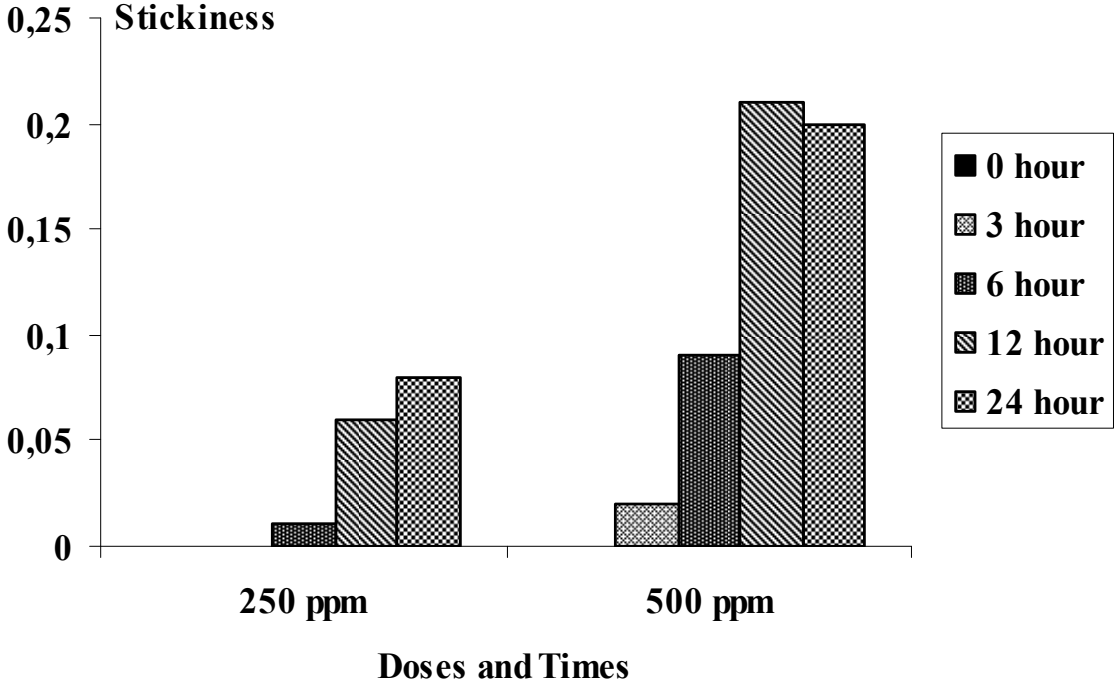

Figure 2. The frequencies sticky metaphases in *A. cepa* rot tip treated with DNOC

The results showed the cytotoxic and genotoxic effect of DNOC in the cell of *A. cepa* L. The MANOVA analysis demonstrated a positive correlation of time and concentration, the effect of the latter being more drastic. The mitotic arresting activity of DNOC was manifested in a high number of cells blocked at mitosis at concentration of 250 ppm, in that substantial increase in the MI was observed. Strong inhibition of mitosis by DNOC, at the highest concentration and treatment time, showed evidence of cytotoxicity. Generally, a mitotic poison causes disturbance of the spindle apparatus, resulting in c-mitosis effects, which means complete absence of a spindle. The results of our study are presented in Fig 3 and demonstrate that DNOC act as a spindle poison in the roots of *A. cepa*.

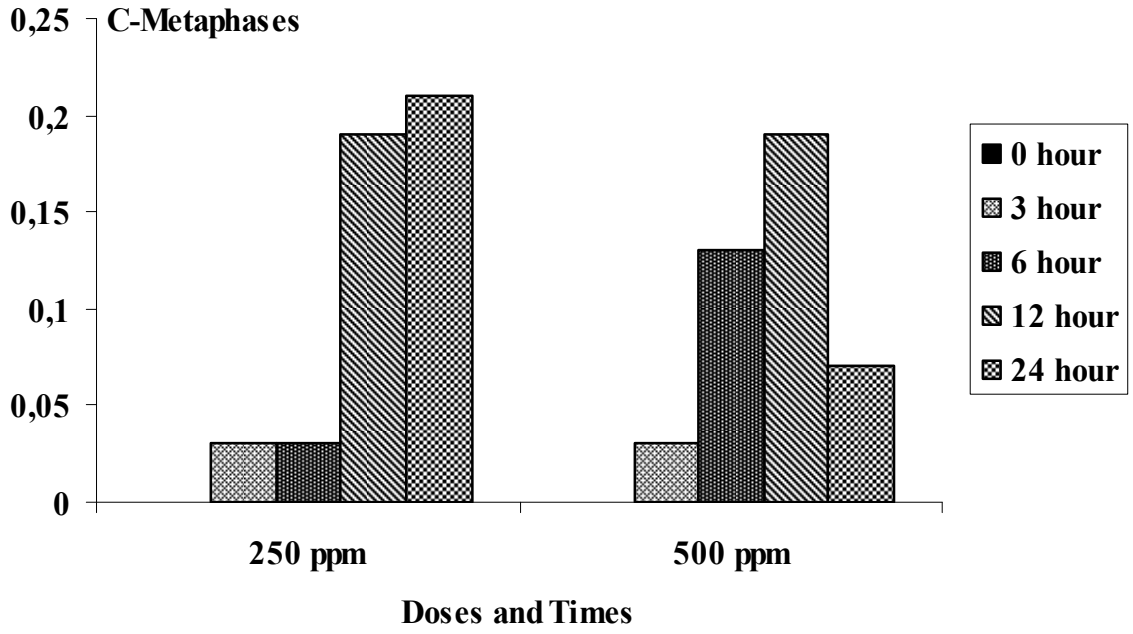

Figure 3. The effect of DNOC on C-metaphases in *A. cepa* root tip cell

The *Allium* test has proved to be reliable test for monitoring cyto- and genotoxicity of the different chemical substances. For in situ monitoring, meristematic cells of *Allium* and *Vicia* were very efficient cytogenetic material for detection of mutagenic activity of the environmental chemicals (Ma et al 1995; Ateeq et al 2002). It had been suggested that plant chromosome analysis might be helpful in cancer research (Levan 1951).

CONCLUSION

According to this genotoxicity results, DNOC is a strong spindle poison and has a clastogenic effect. The *Allium* test presented here is therefore, for many reasons, useful for rapid screening of herbicides that may be health hazardous to humans. Genetic damage in workers may increase at the population level due to occupational exposure to DNOC, under conditions of lack of safety devices and improper handling practices. So, workers should be avoided of careless using.

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