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Araştırma Makalesi / Research Article

Genotoxic Evaluation of Bentazone and Chloridazon Herbicides in *Eisenia hortensis* Coelomocytes

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Abstract

Keywords
Bentazone;
Chloridazon;
Eisenia hortensis;
Genotoxicity;
Comet assay;
Micronucleus

In current study, genotoxic effects of Bentazone and Chloridazon herbicides on *Eisenia hortensis* species were investigated. The species of *E. hortensis* were collected from the natural habitats in Afyonkarahisar. Comet assay and Micronucleus test was conducted to evaluate the genotoxicity in earthworm coelomocytes. The LD₅₀ value of Bentazone herbicides was noted as 236 ppm and 76.6 ppm for Chloridazon, respectively. Then LD_{50/2}, LD₅₀ and 2XLD₅₀ concentrations of Bentazone and Chloridazon herbicides were applied to *E. hortensis* for 48 h. Concentration dependent increase in DNA and chromosomal damage was observed (P < 0.05) by both herbicides. Highest DNA damage and micronucleus formation were noticed at highest doses compared to other concentrations and control group. It was concluded that Bentazone and Chloridazon induced DNA damage and chromosomal aberrations failure in *E. hortensis* earthworms.

Eisenia hortensis Sölomositlerinde Bentazone ve Chloridazon Herbisitlerinin Genotoksisitesinin Değerlendirilmesi

Öz

Anahtar kelimeler
Bentazon;
Chloridazon;
Eisenia hortensis;
Genotoksisite;
Comet yöntemi,
Mikronukleus

Bu çalışmada, Bentazone ve Chloridazon herbisitlerinin *Eisenia hortensis* türleri üzerindeki genotoksik etkileri araştırılmıştır. *E. hortensis* türleri Afyonkarahisar'daki doğal yaşam alanlarından toplanmıştır. Solucan sölomositlerinde genotoksisiteyi değerlendirmek için Comet ve Mikronükleus testi yapıldı. Bentazone herbisitlerinin LD50 değeri Chloridazon için sırasıyla 236 ppm ve 76.6 ppm olarak kaydedildi. Daha sonra LD_{50/2}, LD₅₀ and 2XLD₅₀ konsantrasyonlarında Bentazon ve Chloridazon herbisitleri *E. hortensis*'e 48 saat süreyle uygulandı. Her iki herbisitte de DNA'da konsantrasyona bağlı artış ve kromozomal hasar gözlemlendi (P < 0.05). En yüksek DNA hasarı ve kromozomal sapmaları, diğer konsantrasyonlara ve kontrol grubuna kıyasla en yüksek dozlarda tespit edildi. Bentazone ve Chloridazon'un *E. hortensis*'te DNA hasarı ve kromozomal sapmalara neden olduğu sonucuna varılmıştır.

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1. Introduction

To enhance the crop production, pesticides and herbicides are aggressively used to overcome the yield of harmful organisms. These are either directly applied on soil or indirectly deposited through residual effects and not only affect target organisms, but they also have some side effects such as human and animal toxicity. Irrespective of the ill effects of these chemicals, it is not possible

for many countries to reduce the use of pesticides and herbicides (Venkateswara Rao and Kavitha 2007, Reinecke and Reinecke 2007).

Chloridazon is a residual pyridazinone herbicide, used for the control of weeds in beet, Mangels, Red beet, Onions, garlic and for other crops (Ahmadi et al. 2011). Bentazone is a contact herbicide, used to control the weeds in corn, mint, rice and beans etc (Lina et al. 2020). Both

herbicides are commonly used in fields. In agricultural research, there is a serious concern on the issue of soil toxicity due to the irrational use of herbicides.

Many researches already stated the use of earthworms as a model organism for ecotoxicological assessment. Earthworms significantly contribute towards the decomposition of the organic matter, soil formation and nutrient cycling. They have sensitive receptors on their dermis to detect soil toxicity due to different chemicals. Pesticides and herbicides impair the physiological functions of earthworms and lead the morbidity or mortality of these organisms. This lead to their ecological importance in term of sensitive bioindicator of soil toxicity risk assessment, as even at low concentrations of toxins can be monitored by these organisms. (Lanno et al. 2004, Xiao et al. 2006, Castellanos and Hernandez 2007, Lukkari et al. 2004, Gambi et al. 2007). The quantity of earthworms in soil shows the overall health quality of soil ecosystem and safety level of environment (Xiao et al. 2004).

Several toxicological assays have been applied to asses toxicities of pesticides and herbicides on earthworms (Lukkari et al. 2004, Cigerci et al. 2016). However, many herbicides show specific effects on specific target organisms, and there is a little information is available of their toxicological effects on earthworms. Ecotoxicological effects of Chloridazon and Bentazone were explored through comet assay (CA) and micronucleus assay (MN) on earthworms in the current study.

2. Materials and Methods

2.1. Earthworms collection and experimental method

E. hortensis samples were collected from the Afyonkarahisar's natural surroundings. Species was identified by the specified key of taxonomic classification. The chemicals, bentazone and chloridazon were obtained from the commercial market in the liquid formulation, containing 480 g of bentazone and 520 g of chloridazon per liter of their formulations. Series of different concentrations (0, 50, 100, 250, 500 and 1000 ppm) of these chemicals were employed on earthworms

for 48 h. Species-designated soil worms with developed sexual organs and clitellum were placed in groups of 8 in 5 aerated petri dishes. At the end of 48 h, the head of the earthworms were touched 5 times with a wooden stick and the viability control was performed. The results were evaluated by Probit analysis method and lethal dose was determined. The three concentrations ($LD_{50/2}$, LD_{50} and $2XLD_{50}$) were selected of chloridazon and bentazone. These doses were further used to assess the DNA and chromosomal damage for 48 h. All experimental procedures were carried out in the dark at 24 ± 2 °C. In control group of earthworms, distilled water was used. Three replicates were made for each group to validate the results.

2.2. Comet assay (CA)

Comet assay was carried out on the coelomic fluid of the earthworms to assess the DNA damage. Each earthworm was kept in eppendorf tube containing the extrusion buffer to extract the coelomic fluid from the earthworms. Comet assay was performed as described by the Reinecke and Reinecke (2004), and Cigerci et al 2016. After, lysis and electrophoresis, slides were stained with ethidium bromide. Comet score was calculated by the method of Cigerci et al 2016.

2.3. Micronucleus (MN) test

Rest of coelomocytes was further used for MN test, as described by the (Muangphra and Gooneratne 2011). Briefly, potassium chloride 1 ml (KCl) added in the fluid, centrifuged at 1400 rpm for 6 min. Then, fixative I (1 ml) was added and centrifuged for 10 min at 1400 rpm. Then, cells were again centrifuged with fixative II (1ml), at 1400 rpm for 10 min The cells were spread over the wet clean slides and dried for 24 h. Slides were stained for 15-20 min with giemsa stain and fixed with entellan.

Scores of micronuclear cells were calculated as described by the Cigerci et al 2016.

2.4. Statistical analysis

Analysis of variance (ANOVA) was applied by using SPSS version 15.0. Duncan test was used for making comparison of different concentration. Statistical significance cut-off value was kept as $p < 0.05$.

3. Results

Effects of chloridazon in the form of DNA damage are shown in fig. 1. The LD_{50} determined for chloridazon herbicide was found 76.6 ppm. The $LD_{50/2}$, LD_{50} and $2XLD_{50}$ doses were used for comet assays were 38.3 ppm, 76.6 ppm and 153.2 ppm respectively. Highest DNA damage scores were found at the highest concentrations (53.67 ± 6.66) whereas, lowest was observed in the control group. Concentration dependent increase in DNA damage was observed by the chloridazon and it was found statistically significant ($p < 0.05$).

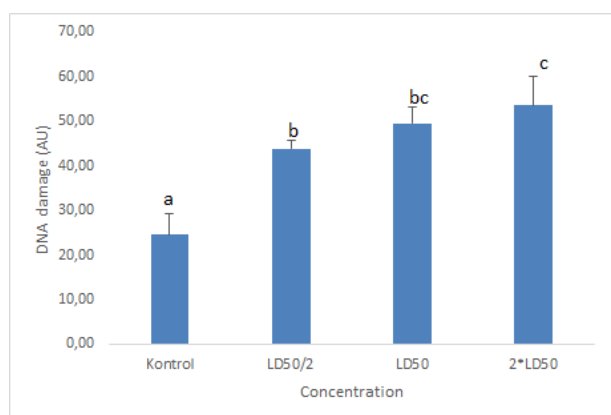


Figure 1. DNA damage caused by the Chloridazon herbicide on *E. hortensis* coelomocytes through comet assay. *The different letters in the columns show significant level at $p < 0.05$.

Similarly, bentazone also showed the dose dependent increase in DNA damage ($p < 0.05$), as shown in Fig 2. The LD_{50} for Bentazone was found at 236 ppm. The DNA damage score at $LD_{50/2}$, LD_{50} and $2XLD_{50}$ doses were found 43.67 ± 2.51 , 51.67 ± 3.05 and 55.33 ± 1.52 respectively.

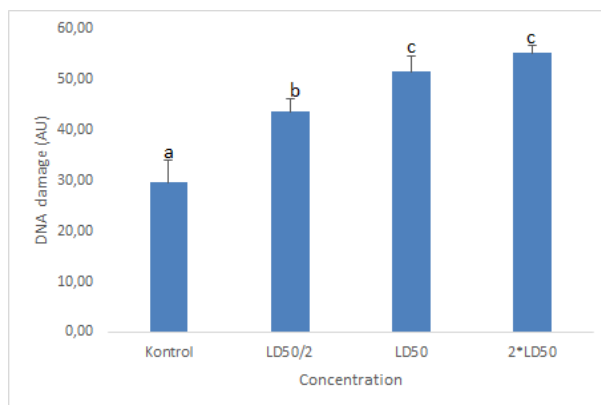


Figure 2. DNA damage caused by the Bentazone herbicide on *E. hortensis* coelomocytes through comet assay. *The different letters in the columns show significant level at $p < 0.05$.

The results of MN are shown in fig 3 and 4. Different concentrations of Chloridazon showed highest chromosomal damage as compared to control group. Highest chromosomal damage was observed at the highest dose whereas, lowest was observed at the lowest concentration. Frequencies of MNi and BN cells were higher in a dose dependent manner and there was significant difference ($p < 0.05$) as compared to control group by the both herbicides.

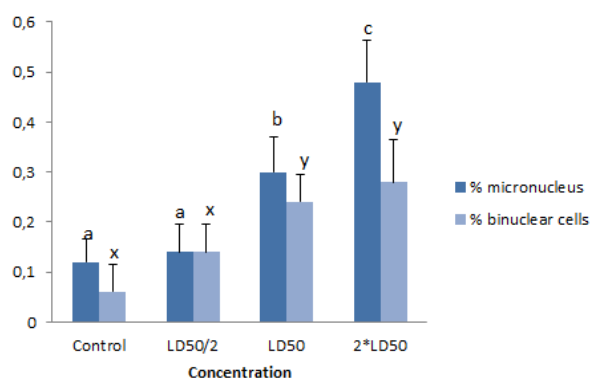


Figure 3. Chromosomal damage caused by the chloridazon herbicide on *E. hortensis* coelomocytes by MN test. *The different letters in the columns show significant level at $p < 0.05$.

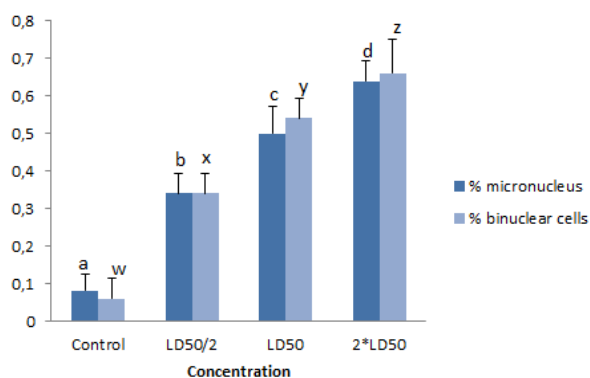


Figure 4. Micronucleus formation caused by the Bentazone herbicide on *E. hortensis* coelomocytes by MN test. *The different letters in the columns show significant level at $p < 0.05$.

4. Discussion

Ecologically, the use of fertilizers and pesticides in agricultural areas is particularly damaging to non-target species due to environmental pollution (Aydin 2006). Many herbicides are being investigated to assess their ecotoxicological effects. But no study has been found to evaluate the genotoxic effects of chloridazon and bentazone by the comet assay and MN test.

Soil worms are particularly used and play an important role in soil ecosystems by improving the physical properties of soil, performing nitrogenation (nitration), helping to form humus, separating and binding organic materials, and ensuring that soil is aerated by the help of tunnels. (Aydin 2006).

In, current study both herbicides showed the genotoxic effects on the earthworm coelomocytes. There was dose dependent increase in the DNA and chromosomal damage by the chloridazon and bentazone.

Bentazone is a member of the thiadiazine group. It is a heterocyclic ring containing nitrogen and sulfur atoms formed as a pair of aromatic rings. It was registered in 1975 and is used as a herbicide after a selective outbreak of weeds, especially with many broad-leaved weeds and cedars. Bentazone showed negative responses to gene mutations in both *in vitro* and *in vivo* systems, chromosome effects, and various tests for primary DNA damage and repair, according to WHO / IPCS and DPR genotoxicity data. Only weak positive responses

have been reported for some clastogenic potential indications in point mutations in Chinese hamster ovary (CHO) cells (HGPRT locus) and in CHO cells with low exposure (Kaya 2004). Dose-related decreases in hemoglobin, hematocrit, and erythrocyte indices in male and female rats were observed by the bentazone along with other low subacute to subchronic toxicity (Brkić et al. 2015). Bentazone also showed the developmental abnormalities against the gold embryo in the time-dependent (Kimmel et al. 1995) and recorded a behavior changes by the goldfish offspring (Saglio et al. 2001).

The chloridazon herbicide showed the DNA damage and MN formation in the coelomocytes. It is thought that chloridazon can interact with DNA through the intercalation mode. In addition, other studies also have been carried out to assess that which base pairs tend to interact with chloridazon. A consistent set of theoretical studies have been shown that using ab initio mechanical quantum to calculate the interaction energies between AT or CG base pairs, chloridazon is tied to the base sequence and that DNA has more interaction with the GC base sequence (Ahmadi 2011).

In current study, both test systems showed the significant results in term of genotoxic assessment of tested chemicals. Comet technique is frequently used in biomonitoring studies because it can even show low level of DNA damage, can be analyzed with fewer cells, can be easily applied, works with different cell and tissue groups, safe and economical (Collins 2003). The micronucleus seen in a cell indicates a numeric or structural genetic damage to that cell. The MN test is a well-known test in human biologic monitoring to detect genotoxicity and detect several nuclear anomalies, micronucleus and binuclear frequencies in coelomocytes, chromosomal defects and inhibition of cytokinesis (Zhan 2012).

An ideal pesticide is defined as "chemical substances that are permanent and devoid of environmental toxicity" that only affect the target organism. Today, however, pesticides pose significant toxicological risks by reaching non-target organisms directly or through residues via soil, water and air. In current study, bentazone and

chloridazon herbicides demonstrated the significant genotoxicity in earthworm coelomocytes. These herbicides are widely used. So, these should be used by the skilled persons and at the appropriate doses to avoid its health hazards effects.

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