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EFFECTS OF α-CYPERMETHRIN PESTICIDE ON DNA STABILITY AND OXIDATIVE ENZYMES IN MAIZE (*Zea mays*)

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Abstract

α-cypermethrin is a pyrethroid and environmental genotoxic pesticide extensively used worldwide for agricultural applications. However, like other pesticides, it affects non-target organisms as well as target organisms and causes various toxic effects. To evaluate α-cypermethrin toxicity in non-target organisms, the effect of α-cypermethrin on the activity of malondialdehyde (MDA, an indicator of lipid peroxidation) content, antioxidant enzymes (superoxide dismutase, SOD; peroxidase, POX and catalase, CAT) and DNA damage were investigated in maize. For this purpose, maize seedlings were exposed to α-cypermethrin in different concentrations (5, 10, 25, and 50 ppm) for 7 days in Petri dishes. After the incubation root length, antioxidant enzyme levels, MDA content and RAPD profiles of seedlings were analyzed to determine toxicity. The results revealed that both MDA content and the activity of antioxidant enzymes (except for 5 ppm at POX application) significantly increased ($p<0.05$) by exposing to α-cypermethrin in a concentration dependent manner. Conversely, significant changes in RAPD profiles were observed after α-cypermethrin applications. These changes included loss of normal bands and the appearance of new bands, in comparison with the control group, and they also were dose-dependent. According to these obtained results, we concluded that α-cypermethrin cause DNA damage in a dose-dependent manner and the root cells of maize exhibits defense against α-cypermethrin-induced oxidative stress by enhancing their antioxidant activities.

Key words: α-cypermethrin, catalase, malondialdehyde, peroxidase, RAPD, superoxide dismutase

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1. Introduction

The total amount of produced pesticides and the extent of pesticide applied agricultural lands have increased all around the world at a faster rate than in the previous 5 decades. It was estimated that the annual consumption of pesticides is approximately two million tons worldwide (Uggini et al., 2010). While pesticide consumption in developed and industrialized countries has decreased in recent years, growing rapidly in developing countries and pesticide addiction increased in these areas. For example, in Turkey, pesticide consumption increased more than twofold during the last decade. The average annual consumption of pesticides in the country is about 35,000 tons and costs about 250 million dolars. The widespread uses of pesticides in agricultural programs often cause environmental pollution and health hazards, including cases of severe, acute and chronic poisoning. The World Health Organization reported that roughly 3 million pesticide poisonings occur annually and result in 20.000 deaths worldwide (WHO, 1992). Conversely, low-level long-term exposure to pesticides may lead to cellular changes, various types of cancer and alterations in the genetic material (Giri et al., 2002).

α-cypermethrin, which is a type II pyrethroid insecticide, is extensively used to control many pests, including moth pests of cotton, fruits, corn and vegetable crops (Suvetha et al., 2010). During the last decade, α-cypermethrin has emerged as a major agricultural pesticide in India, USA, Europe, China and other parts of the world such as Turkey because of its superior insecticidal activity, easy biodegradability,

wide spectrum and relatively moderate mammalian toxicity (Ansari et al., 2011; Kocaman and Topaktas, 2010; Undeger and Basaran, 2005). However, like other insecticides, α-cypermethrin effects non-target organisms in addition to target organisms and causes various toxic effects (Bendahou et al., 1999; el-Tawil and Abdel-Rahman, 1997). According to the US Environmental Protection Agency α-cypermethrin is classified as a toxicity class II chemical (USEPA, 1989). It is highly toxic to fish, bees and aquatic insects and the maximum allowable concentration are 1 ng L^{-1} (USEPA, 1997).

The genotoxic potential of α-cypermethrin has been previously determined by micronucleus test, comet assay and chromosome aberration tests (Ansari et al., 2011; Kocaman and Topaktas, 2010). α-cypermethrin causes induction of DNA damage and micronucleus *in vitro* in human lymphocytes (Undeger and Basaran, 2005). In mouse cells *in vitro*, the pesticide inhibits cell growth and protein synthesis by affecting the DNA (Patel et al., 2006). Shukla et al. (2002) reported that α-cypermethrin was found to have carcinogenic activity in both sexes of Swiss albino mice. It was reported that α-cypermethrin is a peroxisome proliferator that induces mitotic and meiotic abnormalities both *in vivo* and *in vitro*, especially in human and fish cells (Ansari et al., 2011; Kacaman and Topaktas, 2009). Similarly, it causes an increase in chromosomal aberrations, multipolar cells and a decrease in mitotic index in *Allium cepa* (Inceer et al., 2009). Furthermore, some recent studies using the method of *in vivo* and *in vitro* sister chromatid exchange (SCE) provide evidence of genetic activity of commercial α-cypermethrin (Giri et al., 2003; Kocaman and Topaktas, 2009). Although its toxicity to animals has been studied in recent years, little information is available on its effects on plants. Some pesticides cause mutagenic activity and alter the metabolism of antioxidative enzymes in certain plant species. When plants are exposed to pesticides they often suffer oxidative stress caused by the generation of reactive oxygen species (ROS) such as superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical (•OH), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2) (Salin, 1988). Plants have developed removal apparatus for protection from the detrimental impacts of ROS on the system that contains molecules and enzymes (i.e. CAT, POX, and SOD) having an antioxidative capacity (Scandalios, 1993; Sun et al., 2008).

Improvements in various PCR-based methods proceed with the developments in molecular biology were used for investigation of DNA in several research areas, such as genetic toxicology (Liu et al., 2007). Random amplified polymorphic DNA (RAPD), is a PCR-based method used for amplification of randomly selected DNA sequences with primers shorter than normal under low annealing temperatures. The extensive usage areas of this method involved the classification of species, a formation of genetic maps and determination of evolutionary relationships and origins (Liu et al., 2005; Zhiyia and Haowen, 2004).

In addition, RAPD was in use for the detection of damages and changes on DNA by scanning the whole genomic DNA for that kind of alterations or variations from the simplest organisms to the most advanced organisms (Liu et al., 2005; Liu et al., 2009).

Although α-cypermethrin toxicity is generally known in non-target organisms (especially animals), however, a small number of studies considered its toxic effects on the genetic material of crop plants (Inceer et al., 2009; Saxena et al., 2005). In vitro research related to DNA damage of α-cypermethrin in plant systems is also inadequate. This is the first study reporting the oxidative stress and DNA damage of α-cypermethrin on maize seedlings and the possible mechanism in terms of standard toxicological and molecular parameters.

2. Materials and methods

2.1. Chemicals, growth conditions, antioxidant enzyme activities, and MDA content

Four different concentrations (5, 10, 25 and 50 ppm) of α-cypermethrin were used for genotoxicity assays (Inceer et al., 2009). In the preparation and analysis of the samples, the procedure described in my previous study was followed (Aksakal, 2013). The seeds were sterilized with 75% ethanol for 2 minutes, followed by 10% sodium hypochlorite for 10 minutes and washed with distilled water at least five times. Maize seeds were germinated to primary roots of 2-4 mm length in a sterile glass jar containing three layers of Whatman paper for 48 hours at 25±2°C in the dark. 15 equal germinated seeds were chosen and transferred to 15 diameter sterile Petri plates covered by the aluminum paper to avoid degradation of the pesticide (photosensitive), and treated with different pesticide concentrations (5, 10, 25 and 50 ppm). Plant seedlings were subjected to α-cypermethrin in a climate chamber for a week under identical conditions. Enzymatic activities of antioxidant system were identified 7 days after the end of incubation. For identification of SOD, CAT and POX activities, the roots of maize plants were homogenated in 1 mmolL^{-1} ethylenediaminetatraacetic acid (EDTA) and 1% (w/v) polyvinyl pyrrolidone containing potassium phosphate buffer (pH 7.0). The centrifugation process of the homogenate was performed at 15.000 x g for 15 minutes at 4°C. Then, the soluble part above the precipitate was used as the source of enzymes.

A modified version of the method developed by Agarwal and Pandey (2004) was used to check the total SOD activity. An enzyme activity unit was defined as the amount of enzyme that reduced the absorbance value to 50% compared to the tubes with enzyme deficiency. CAT activity was measured in accordance with the method developed by Havir and McHale (1987). A CAT activity unit was defined as the amount of enzyme that separates 1 μmol H_2O_2 per minute. The method of Ye et al. (2003) was utilized for measuring the POX activity. An 0.01 rise in the

amount of absorbed light per minute by the enzyme was described as the unit of POX activity.

Lipid peroxidation measurement was performed according to the method of Jalel et al. (2007) using the thiobarbituric acid (TBA) test that determines MDA as an end product of lipid peroxidation. Seedling material weighing 0.5 g was homogenized in 3 mL 0.1% trichloroacetic acid (TCA) and centrifuged at 15.000 × g for 30 min at 4°C. One milliliter of reagent (0.5% TBA in 20% TCA, w/v) was added to a 0.5 mL aliquot of the supernatant. For a negative control, 0.5 mL 0.1% TCA and 1 mL reagent were added. The test tubes were heated at 95°C for 30 min and then quickly cooled in an ice bath. After cooling and centrifugation to give a clear supernatant, the absorbance of the supernatant at 532 nm was read and the value of the non-specific absorption at 600 nm was subtracted. The amount of MDA-TBA complex (red pigment) was calculated from the extinction coefficient 155 (mmol $L^{-1})^{-1}$ cm^{-1}.

2.2. Genomic DNA isolation

Genomic DNA was extracted using the procedure reported by Aksakal et al. (2013) with minor modifications. Approximately, 10-15 mg of fresh root sample that was powdered in liquid nitrogen measured and added to a 2 ml tubes. 1 ml extraction buffer containing, 100 mM Tris-HCl; 50 mM EDTA; 500 mM NaCl; 2% SDS (w/v); 2% β-mercaptoethanol (v/v); 1% PVP (w/v) was added, and the solution was mixed by inverting tubes. The tubes were then incubated in a water bath pre-heated to 65ºC. The tubes were kept in the water bath for approximately 45-60 minutes and mixed by inverting tubes at 10-minute intervals. After the bath, the samples were centrifuged at 14000 rpm for 15 minutes at 4ºC. 600 µL of the supernatant was carefully transferred into a clean 1.5 ml tube and mixed with 600 µL volume of phenol-chloroform isoamyl alcohol, and then centrifuged again in the same conditions. The supernatant was transferred in a clean tube and 60 µL hot 10% CTAB-0.7M NaCl was added and centrifuged again (14.000 rpm for 15 minutes). Then, the supernatant was transferred into another clean tube and an equal volume of chloroform:isoamyl alcohol (24:1) was added and mixed gently. After this step, 0.6 volume of isopropanol (must be cold) was added, and the samples were placed in a refrigerator (-20ºC) for 10-20 minutes. By the last centrifugation (14.000 rpm for 15 minutes) DNA was collected at the bottom of the tube, and the isopropanol was poured off carefully (this stage is sensitive); air-dry DNA samples dissolved in 100-150 µL of TE buffer. The density of DNA samples was assessed in 1% agarose gel electrophoresis and the concentration was determined using ultra spectral analysis.

2.3. RAPD Procedures

Standard 10-base primers supplied by operon (Operon Technologies Inc., Alameda, CA, USA) were used to screen RAPD variation. The PCR mixture (30 µL) was prepared as follows: 3.0 µL of 10x PCR buffer, 1.2 µL of dNTPs (10 mM), 1.2 µL of magnesium chloride (25 mM), 2.0 µL of primer (5 µM), 0.4 µL polymerase enzyme (Taq) (5 unit), 19.2 µL distilled water, 3.0 µL of DNA sample (100 ng/µL). Forty oligonucleotide primers were screened, and among them, 16 primers were selected and used for further studies. Sequences (5'→3') of primer 1 to 16 are CACTCTCCTC (OPH-17), CAGGCCCTTC (OPA-1), GTCCTGGGTT (OPW-17), CTGATGCGTG (OPW-11), TTCAGGGCAC (OPW-18), AATCGGGCTG (OPA-4), CTGACCAGCC (OPH-19), GGGTCTCGGT (OPY-13), ACCAGGTTGG (OPH-14), GAATCGGCCA (OPH-18), GTGGCATCTC (OPY-1), AGGCAGAGCA (OPY-8), AGTCGCCCTT (OPY-15), GTCCACACGG (OPB-8), CTCAGTGTCC (OPW-1), GGCGGATAAG (OPW-5), respectively.

The PCR conditions were: 2 minutes at 95°C; 2 cycles of 30 seconds at 95°C, 1 minute at 37°C, 2 minutes at 72°C; 2 cycles of 30 seconds at 95°C, 1 minute at 35°C, 2 minutes at 72°C; 41 cycles of 30 seconds at 94°C, 1 minute at 35°C, 2 minutes at 72°C; then a final extension at 72°C for 5 minutes. Finally, the samples were cooled to 4°C.

2.4. Agarose gel electrophoresis

After the PCR process, the products (27 µL) were mixed with 3 µL of 6x gel loading dye and loaded to the agarose (1.5% w/v including 2 µL EtBr) gel electrophoresis in 0.5xTBE (Tris-Borate- EDTA) buffer. The electrophoresis conditions were at 70 V for 150 minutes. Bio Doc Image Analysis System with Uvisoft analysis package (Cambridge Electronic Design Ltd, Cambridge, UK) was used for the detection of amplified DNA product. Total Lab TL120 computer software was used for the evaluation of RAPD patterns.

We have calculated Genomic Template Stability (GTS, %) using the following formula (Eq. 1):

$$GTS = (1 - \frac{a}{n}) x100 \quad (1)$$

where *a* is the total polymorphic bands counted in each treated sample and *n* is the total bands in the control. Polymorphic bands were comprised of missed bands and/or the appearance of new bands against the control. The GTS value was checked for each α-cypermethrin treated sample. The GTS value of the control was set to 100% and the treated samples were calculated according to the control.

2.5. Statistical analysis

All given data are the representative values of the means. The computed mean ± SE values were used in showing the data of at least six experiments. One-

way analysis of variance and Duncan's multiple-range tests were used to compare the mean values with a significance of 5%.

3. Results

3.1. Effects of α-cypermethrin on root length, antioxidant enzymes activities and MDA

The length of the roots reduced compared to control seedlings with increased concentration of α-cypermethrin (Table 1). Root growth was inhibited more than 50% in maize seedlings when applied with 25 and 50 ppm α-cypermethrin concentrations. A change in the overall morphology of the root system was also observed. Root hair growth was reduced and the distance between the apex and the root hair zone decreased. The alterations in SOD, CAT and POX enzymatic activities were quantified in maize seedlings for the evaluation of the variations in antioxidative defense mechanisms after α-cypermethrin applications. SOD and CAT activities in all α-cypermethrin treatment groups increased when compared with the control group in a dose-dependent manner (Table 1). Furthermore, α-cypermethrin treatments (except for 5 ppm) caused increases in POX activity in maize seedlings when compared with the control groups (Table 1) and these increases were statistically significant ($p<0.05$). As a result of the elevated level of the antioxidant activity, MDA content was also increased in the seedlings treated with α-cypermethrin compared to the control group (Table 1).

3.2. Effects of α-cypermethrin on RAPD profiles and DNA stability

Totally, forty primers consisting of 10 nucleotides were used to detect amplification products for PCR experiments. Just 16 of them gave distinct and invariable bands with a number of 111. The summary of all RAPD profile changes and RAPD products of 8 chosen primers were shown in Table 2 and Fig. 1, respectively. Each primer generated 4 to 10 bands with an average of 6.9 bands per primer. The amplification size of the PCR products in control seedlings ranged from 123 pb to 894 pb. RAPD profiles varied significantly between control and α-cypermethrin treated seedlings in terms of the obvious variations in the number and size of DNA fragments amplified with different primers.

Table 1. Effects of α-cypermethrin on antioxidant enzyme activities, MDA content and root lengths in the maize seedlings

Concentration	***Root Length Cm***	***SOD U.mg^{-1}***	***CAT U.mg^{-1}***	***POX U.mg^{-1}***	***MDA nmol.mL^{-1}***
Control	11.0^{a}	53.4^{a}	15.7^{a}	2042^{a}	0.67^{a}
5 ppm	9.1^{b}	60.3^{b}	77.7^{b}	1869^{b}	0.81^{b}
10 ppm	7.4^{c}	73.9^{c}	79.2^{b}	2100^{a}	0.83^{b}
25 ppm	4.9^{d}	85.8^{d}	89.5^{c}	2568^{c}	1.24^{c}
50 ppm	3.0^{e}	116.8^{e}	89.9^{c}	3046^{d}	1.35^{c}

Data are means of at least three determinations with two replicates
^{a,b,c,d,e} Different letters in the same column indicate statistically significant differences $p<0.05$

Table 2. The number of bands in the control and disappearance (-), and/or appearance (+) of DNA bands with molecular sizes (base pair, bp) for all the primers of α-cypermethrin treated maize seedlings ND: Non detected

Primer	***Cypermethrin concentration***				
	Control	5 ppm	10 ppm	25 ppm	50 ppm
OPH-17	6	ND	ND	+423,+695	ND
OPA-1	6	ND	+355	-270,-320,-467	-467
OPW-17	7	ND	ND	+320	-238
OPW-11	9	ND	-360,+285,+623	-342,-360,+623	-342,-360,-415,-577
OPW-18	7	-872,+377	-255,-872	-872,+123,+200	-872
OPA-4	7	ND	ND	-123,-894,+272,+305	-123,-358,-894 +272,+305,+480
OPH-19	6	-200,-324,-415,-460,-610	-324,-460,+152,-610	-324	-324,+152
OPY-13	7	ND	-430,-651	-430,-651	-322,-376,-395,-430,-507, -651
OPH-14	9	+525,+553,+587,+620	+525,+553,+620	-175,+620	-260, +525,+553,+620
OPH-18	6	ND	-300,+175	-300,-385,+175,+610	-300,-385,-410+175
OPY-1	8	ND	+410	-250	-250
OPY-8	5	+510,+600	+510,+570,+600	+510,+570,+600,+700	+510,+570,+600
OPY-15	4	+520	-223,+520	-223,+520	-223,-300+520
OPB-8	10	ND	-155,-200,-250,	ND	-534,-623
OPW-1	5	+155	+400	-215	-215
OPW-5	9	+410,+522	+410,+522	+410,+522	-580,-710,+410,+522

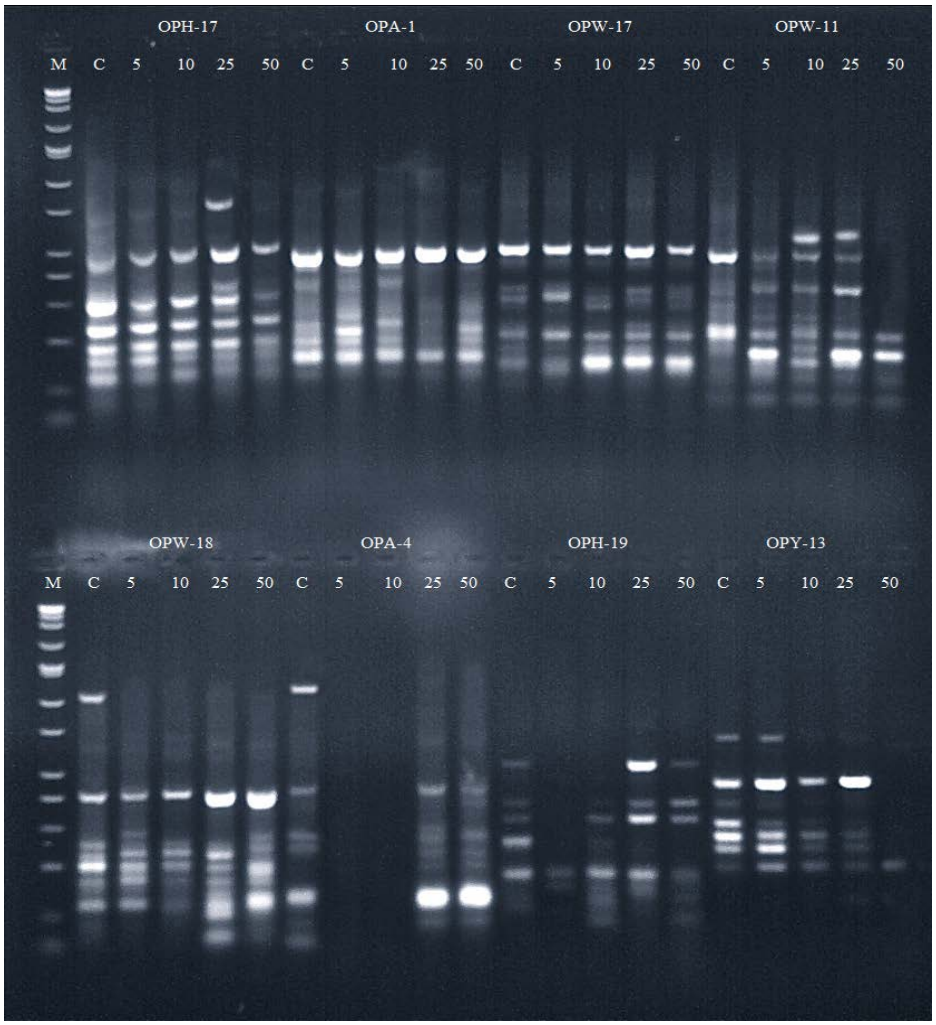

Fig. 1. RAPD profiles of 8 primers of genomic DNA extracted from maize seedlings exposed to α-cypermethrin for 7days

The main detections or alterations observed in RAPD profiles produced by seedlings treated with α-cypermethrin were the emergence of new bands and/or loss of normal bands compared to control ones. These variations have been observed in many of the RAPD profiles. Some of the primers, however, have altered a remarkable number of amplification products (OPH-17, OPW-17, OPY1), whereas the others (OPH-14, OPH-19) have given more complex appearance or disappearance of bands. A total of 40 normal RAPD bands observed in control seedlings got lost after the treatment of α-cypermethrin. In addition, 30 new bands have emerged in every α- cypermethrin applications. Different polymorphic bands were defined for different primers at either concentration of α-cypermethrin. The polymorphism rate was P%= 17.5%, 28.9%, 34.3%, 39.8% for 5, 10, 25 and 50 ppm α-cypermethrin, respectively. Polymorphisms originated from the loss or emergence of amplification bands in the treatment profiles compared to control ones. For every 16 primers, the genomic template stability (a measurable value for reflection of the alterations in RAPD patterns) was calculated and shown in Table 3. As demonstrated in Table 3, the average GTS rates declined significantly with the rise in the concentration of α- cypermethrin.

4. Discussions

In addition to the increase in membrane lipid peroxidation, a rapid rise in the amount of MDA is observed when the plants are exposed to oxidative stress. In this study, high concentrations of α-cypermethrin (25-50ppm) were observed to increase the MDA content in maize compared to control group, and a large amount of lipid peroxidation was observed in treated seedlings of maize. The increase in lipid peroxidation upon exposure to α-cypermethrin may be attributed to the inductions of reactive oxygen species (ROS) in cells. It is well known that ROS contain superoxide anion radical ($O_2^{\bullet -}$) produced in the cytosol, mitochondria and endoplasmic reticulum, hydrogen peroxide (H_2O_2) produced in peroxisomes, the highly reactive hydroxyl radical (•OH) and singlet oxygen ($^1O_2$).

Important cellular components such as DNA, proteins, and lipids can be significantly affected by the oxidative damage that may cause by ROS (Lin et al., 2008). Plants, like many other organisms, have unique systems that protect themselves against the deleterious effect of elevated ROS. Different cellular compartments for the reduction of the excess amount of ROS can activate various defense systems.

Table 3. Changes of GTS all primers of α-cypermethrin-contaminated maize seedlings for 7 days

Primer	***Cypermethrin concentrations***				
	Control	**5 ppm**	**10 ppm**	**25 ppm**	**50 ppm**
OPH-17	100	100	100	66.6	100
OPA-1	100	100	83.8	50	83.8
OPW-17	100	100	100	85.7	85.7
OPW-11	100	100	66.6	66.6	55.5
OPW-18	100	71.4	71.4	57.1	85.7
OPA-4	100	-	-	42.8	14.2
OPH-19	100	16.6	33.3	83.3	66.6
OPY-13	100	100	71.4	71.4	14.2
OPH-14	100	55.5	66.6	77.7	55.5
OPH-18	100	100	66.6	33.3	33.3
OPY-1	100	100	87.5	87.5	87.5
OPY-8	100	60	40	20	40
OPY-15	100	75	50	50	25
OPB-8	100	100	70	100	80
OPW-1	100	80	80	80	80
OPW-5	100	77.7	77.7	77.7	55.5
Average	100	82.4	71.0	65.6	60.1

These protection systems contain non-enzymatic proteins (e.g. transferrin, ferritin), oxidizable molecules, trace elements (e.g. selenium, zinc), and particularly antioxidant enzymes (e.g. CAT, POX, and SOD), which are seen as biological markers of oxidative stress.

Among antioxidant enzymes, SOD is a major enzyme that contributes to the defense against oxidative stress by converting superoxide radicals ($O_2^{\bullet-}$) to O_2 and H_2O_2 at a very fast rate and contributes to defense against oxidative stress (Mittler, 2002). Therefore, with the increase in SOD activity, endogenous H_2O_2 production observed as a result of the application of α-cypermethrin was also increased. In this study, elevated SOD activity was observed in every α-cypermethrin applied maize plants. The elevated amount of SOD activity was probably due to both the direct impact of α- cypermethrin and the indirect impact of the rise in the superoxide radical levels. High levels of SOD activity can also be explained by the fact that ROS is responsible for the activation of genes that encode antioxidant enzymes such as SOD (Holgrem, 2003). The results of the present experiment are in agreement with the findings of Kale et al. (1999), who observed an increase in SOD activity in α-cypermethrin treated rat erythrocytes.

Apart from SOD, other antioxidant enzymes such as POX and CAT are found both in prokaryotes and eukaryotes, which are believed to be very effective in antioxidant defense mechanisms. POX is an enzyme related to plant development, growth and senescence processes, resistance to pathogens, wound healing, lignin and ethylene synthesis and biological reduction of IAA (Asada, 1992). This enzyme could play a role in the reduction of oxidative stress caused by toxic substances via catalyzing the oxidation of the substrate bound to H_2O_2. The other important enzyme in the antioxidant system is CAT and it helps to remove the ROS derivatives from plant cells. CAT can have a role in the control the H_2O_2 level in plant cells by joining the main defense against H_2O_2 accumulation and toxicity. In current experimental conditions, POX and CAT activities in concentrations of 10-50 ppm showed a rising trend. Similarly, Jin et al. (2011) observed a significant increase in CAT and POX activities in male mice after exposure to 20 mg kg^{-1} α-cypermethrin for 21-42 days. It can be said that the significant increase in the levels of SOD, CAT, and POX activities in maize seedlings at given α-cypermethrin concentrations and duration demonstrate efficient antioxidative defense against α-cypermethrin-induced oxidative stress. MDA, SOD, POX and CAT activities also increased in fish liver cells exposed to similar concentrations of α-cypermethrin (Uner et al., 2001).

The extent to which the genotoxic chemicals cause changes in the structure of DNA can be determined at both biochemical and molecular levels by using a variety of biomarker tests. In this study, analysis of root length and changes in the amount of antioxidant enzymes that provide biochemical information were presented in Table 1. At the same time, negative effects of α-cypermethrin on maize at molecular level were evaluated by the RAPD technique. Because of its reliability, sensitivity and reproducibility and having the potential to detect diverse amount of DNA damage as well as mutations, it is frequently used in eco-toxicological research. RAPD analysis was efficiently used to determine the negative effects of pesticides as well as some toxic heavy metals on DNA (Aksakal et al., 2013; Bozari and Aksakal, 2012; Oztürk et al., 2010). According to our results, 5 to 50 ppm α-cypermethrin induced genotoxic damage in the early life stage of maize seedlings. The application of α-cypermethrin at certain concentrations has been shown to limit the mitotic activity and cell division by altering some chemical reactions of the plants (Inceer et al., 2009). It is believed that these changes in chemical reactions occur as a result of the interaction of α-cypermethrin

with membrane phospholipids and its enzyme binding properties. In addition, this pesticide leads to some particular alterations in DNA structure and/or cell functions by binding to proteins and lipids and it may also cause the free radical formation (Inceer et al., 2009; Jin et al., 2011; Patel et al., 2006). The present results are in good agreement with the outcomes of the earlier studies mentioned above.

A total of 111 bands were obtained by using 16 primers containing 10 nucleotides in RAPD analysis. Every primer consisted of 4 to 10 bands with an average of 6.9 bands per primer. Aksakal et al. (2013) applied 16 decamer primers to evaluate the genotoxic effects of 2,4-D on maize and found an average of 7.2 bands per primer. Bozari and Aksakal (2012) applied 15 decamer primers to study the genotoxic effects of trifluralin on maize and found an average of 6.0 bands per primer. It was reported that toxicant-induced genetic changes might arise from the direct action of the toxicant at the DNA level or from indirect actions (Bozari and Aksakal, 2012). In the present study, with the increases of α-cypermethrin doses, an increase was also obtained in polymorphism value and a decrease in GTS. These affects may arise from the replication-preventive feature of the chemical.

Alternatively, the differences in DNA profiles observed in the present study could be attributed to the changes in oligonucleotide priming sites that mainly depend on complex chromosomal rearrangements and are less likely the result of point mutations or DNA damage (e.g., single- and double-strand breaks, abasic sites, DNA-protein cross-links and modified bases) in the primer-binding sites or the presence of DNA photoproducts, which can block or reduce the polymerization of DNA in the PCR reaction for the disappearance of normal bands and mutation (i.e., new annealing events), large deletions (i.e., bringing closer to preexisting annealing site) and homologous recombinations for the appearance of new bands (Aksakal et al., 2013).

Previous studies revealed that changes in the RAPD profiles caused by genotoxins could also be seen as alterations in the GTS and similar genotoxic effects can be directly compared with the changes in certain parameters such as root growth (Aksakal et al., 2013; Bozari and Aksakal, 2012). The results revealed that the GTS was influenced by α-cypermethrin concentrations, which was further correlated with decreased root growth. An identical result in GTS of maize seedlings has also been notified due to the use of trifluralin and 2,4-D (Aksakal et al., 2013; Bozari and Aksakal, 2012). RAPD technique has been reported to be more sensitive than classical genotoxic tests (Liu et al., 2007; Ozturk et al., 2010). Because RAPD analysis was able to detect temporary DNA changes that did not emerge as mutations at the end. The existing results of the α-cypermethrin exposure in *Zea mays* seedlings are in accordance with the previous studies on other organisms. As a result, the RAPD method can be used as a research tool for genomic changes induced by α-cypermethrin. In addition, current results imply that RAPD may be a useful strategy to evaluate the genotoxicities of pesticides along with physiological parameters.

5. Conclusions

In the present study, the toxic effects of α-cypermethrin on maize were discussed. The concentrations of α-cypermethrin at low and high levels increased the antioxidant enzyme activities and MDA content and decreased the root length. Upon exposure to α-cypermethrin, the alterations in RAPD profiles of maize seedlings presented modifications in the appearance or disappearance of bands compared to the control groups.

A significant negative correlation between GTS and elevated amounts of α- cypermethrin was clearly noticed. In conclusion, the data obtained from the present study indicates that α-cypermethrin, like other pesticides in the environment, can be obtained by crops and may adversely affect their cells and genomes, therefore, causing damage to plants.

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References

Agarwal S., Pandey V., (2004), Antioxidant enzyme responses to NaCl stress in *Cassia angustifoli*, *Biologia Plantarum*, **48**, 555-560.

Aksakal O., (2013), Assessment of paraquat genotoxicity on barley (*Hordeum vulgare* L.) seedlings using molecular and biochemical parameters, *Acta Physiologiae Plantarum*, **35**, 2281-2287.

Aksakal O., Erturk F., Sunar S., Bozari S., Agar G., (2013), Assessment of genotoxic effects of 2,4-dichlorophenoxyacetic acid on maize by using RAPD analysis, *Industrial Crops and Products*, **42**, 552-557.

Ansari R.A., Rahman S., Kaur M., Anjum S., Raisuddin S., (2011), In vivo cytogenetic and oxidative stress inducing effects of cypermethrin in freshwater fish, *Channa punctata* Bloch, *Ecotoxicology and Environmental Safety*, **74**, 150-156.

Asada K., (1992), Ascobate peroxidase a hydrogen peroxide scavenging enzyme in plants, *Physiologia Plantarum*, **85**, 235-241.

Bendahou N., Fleche C., Bounias M., (1999), Biological and biochemical effects of chronic exposure to very low levels of dietary cypermethrin (Cymbush) on honeybee colonies (Hymenoptera: Apidae), *Ecotoxicology and Environmental Safety*, **44**, 147-153.

Bozari S., Aksakal O., (2012), Application of random amplified polymorphic DNA (RAPD) to *detect* genotoxic effect of trifluralin on maize (*Zea mays*), *Drug & Chemical Toxicology*, **36**, 163-169.

el-Tawil O.S., Abdel-Rahman M.S., (1997), Effect of cypermethrin on isolated male and female rat hepatocytes, *Journal of Toxicology and Environmental Health*, **52**, 461-474.

Giri S., Prasad S.B., Giri A., Sharma G.D., (2002), Genotoxic effects of malathion: an organophosphorus insecticide, using three mammalian bioassays *in vitro*, *Mutation Research*, **54**, 223-231.

Giri S., Giri A., Sharma G.D., Prasad S.B., (2003), Induction

of sister chromatid exchanges by cypermethrin and carbosulfan in bone marrow cells of mice in vivo, *Mutagenesis*, **18**, 53-58.

Inceer H., Hayıroglu-Ayaz S., Ozcan M., (2009), Genotoxic effects of the insecticide cypermethrin on the root meristem cells of sunflowers (*Helianthus annuus* L.), *Bulletin Environmental Contamination and Toxicology*, **83**, 652-656.

Havir E.A., McHale N.A., (1987), Biochemical and development characterization of multiple forms of catalase in tobacco leaves, *Plant Physiology*, **84**, 450-455.

Holgrem A., (2003), *Redox Regulation of Genes and Cell Function*, In: *Critical Reviews of Oxidative Stress and Aging: Advances in Basic Science, Diagnostics and Intervention, Band 1*, Cutler R.G., Rodriguez H. (Ed.), World Scientific Press, 103-112.

Jalel C.A., Manivannan P., Sankar B., Kishorekumar A., Panneerselvam R., (2007), Calcium chloride effects on salinity induced oxidative stress, proline metabolism and indole alkaloid accumulation in *Catharanthus roseus*, *Comptes Rendus Biologies*, **330**, 674-683.

Jin Y., Zheng S., Pu Y., Shu L., Sun L., Liu W., Fu Z., (2011), Cypermethrin has the potential to induce hepatic oxidative stress, DNA damage and apoptosis in adult zebrafish (*Danio rerio*), *Chemosphere*, **82**, 398-404.

Kale M., Rathore N., John S., Bhatnagar D., (1999), Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species, *Toxicology Letters*, **105**, 197-205.

Kocaman A.Y., Topaktas M., (2009), The in vitro genotoxic effects of a commercial formulation of α-cypermethrin in human peripheral blood lymphocytes, *Environmental and Molecular Mutagenesis*, **50**, 27-36.

Kocaman A.Y., Topaktas M., (2010), Genotoxic effects of a particular mixture of acetamiprid and α-cypermethrin on chromosome aberration, sister chromatid exchange, and micronucleus formation in human peripheral blood lymphocytes, *Environmental Toxicology*, **25**, 157-168.

Lin A., Zhang X., Zhu Y.G., Zhao F.J., (2008), Arsenate-induced toxicity: effects on antioxidative enzymes and DNA damage in *Vicia faba*, *Environmental Toxicology and Chemistry*, **27**, 413-419.

Liu W., Li P.J., Qi X.M., Zhou Q.X., Zheng L., Sun T.H., Yang Y.S., (2005), DNA changes in barley (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD analysis, *Chemosphere*, **61**, 158-167.

Liu W., Yang Y.S., Zhou Q., Xie L., Li P., Sun T., (2007), Impact assessment of cadmium contamination on rice (*Oryza sativa* L.) seedlings at molecular and population levels using multiple biomarkers, *Chemosphere*, **67**, 1155-1163.

Liu W., Yang Y.S., Li P.J., Zhou Q.X., Xie L.J., Han Y.P., (2009), Risk assessment of cadmium-contaminated soil on plant DNA damage using RAPD and physiological indices, *Journal of Hazardous Materials*, **161**, 878-883.

Mittler R., (2002), Oxidative stress, antioxidants, and stress tolerance, *Trends in Plant Sciences*, **7**, 405-410.

Ozturk F., Duman F., Leblebici Z., Temizgul R., (2010), Arsenic accumulation and biological responses of watercress (*Nasturtium officinale*) exposed to arsenite, *Environmental and Experimental Botany*, **69**, 167-174.

Patel S., Pandey A.K., Bajpayee M., Parmar D., Dhawan A., (2006), Cypermethrin-induced DNA damage in organs and tissues of the mouse: Evidence from the comet assay, *Mutation Research*, **607**, 176-183.

Saxena P.N., Chauhan L.K.S., Gupta S.K., (2005), Cytogenetic effects of commercial formulation of cypermethrin in root meristem cells of *Allium sativum*: spectroscopic basis of chromosome damage, *Toxicology*, **216**, 244-252.

Salin M.L., (1988), Toxic oxygen species and protective systems of the chloroplast, *Physiologia Plantarum*, **72**, 681-689.

Scandalios J.G., (1993), Oxygen stress and superoxide dismutases, *Plant Physiology*, **101**, 7-12.

Shukla Y., Yadav A., Arora A., (2002), Carcinogenic and cocarcinogenic potential of cypermethrin on mouse skin, *Cancer Letters*, **182**, 33-41.

Sun Y.Y., Yin Y., Zhang J.F., Yu H.X., Wang X.R., Wu J.C., Xue Y.Q., (2008), Hydroxyl radical generation and oxidative stress in *Carassius auratus* liver, exposed to pyrene, *Ecotoxicology and Environmental Safety*, **271**, 446-453.

Suvetha L., Ramesh M., Saravanan M., (2010), Influence of cypermethrin toxicity on ionic regulation and gill Na^+/K^+-ATPase activity of a freshwater teleost fish *Cyprinus carpio*, *Environmental Toxicology and Pharmacology*, **29**, 44-49.

Uggini G.K., Patel P.V., Balakrishnan S., (2010), Embryotoxic and teratogenic effects of pesticides in chick embryos: a comparative study using two commercial formulations, *Environmental Toxicology*, **27**, 166-174.

Undeger U., Başaran N., (2005), Effects of pesticides on human peripheral lymphocytes in vitro: induction of DNA damage, *Archives of Toxicology*, **79**, 169-176.

Uner N., Oruc E.O., Canli M., Sevgiler Y., (2001), Effects of cypermethrin on antioxidant enzyme activities and lipid peroxidation in liver and kidney of the freshwater fish, *Oreochromis niloticus* and *Cyprinus carpio* (L.), *Bulletin Environmental Contamination and Toxicology*, **67**, 657-664.

USEPA, (1989), Guidelines establishing test procedures for the analysis of pollutants under the clean water. Act Fed Reg Vol 54. USEPA: 50216-50223.

USEPA, (1997), Office of pesticide programs reference dose tracking report. Washington D.C., On line at: http://ace.orst.edu/info/nptn/ tracking/tracking.htm.

WHO, (1992), Our planet, our health: report of the WHO commissions on health and environment, World Health Organization, Geneva, Switzerland.

Ye Y., Tam N.F.Y., Wong Y.S., Lu C.Y., (2003), Growth and physiological responses of two mangrove species (Bruguiera *gymnorrhiza* and *Kandelia candel)* to waterlogging, *Environmental and Experimental Botany*, **49**, 209-221.

Zhiyia R., Haowen Y., (2004), A method for genotoxicity detection using random amplified polymorphism DNA with *Danio rerio*, *Ecotoxicology and Environmental Safety*, **58**, 96-10.