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Cytotoxic and genotoxic assessment of 2-chloropyridine using *Allium cepa* ana-telophase and comet test

2-Kloropiridin'in *Allium cepa* ana-telofaz ve komet testi kullanılarak sitotoksik ve genotoksik değerlendirilmesi

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ABSTRACT

2-Chloropyridine (2-CPY) is an important precursor of cosmetics, pesticides and other pharmaceutical products and it is also defined as trace chemical in industrial wastewater as the products of the metabolites of agricultural chemical products and river pollutants. In this study, the effects of 2-CPY on mitotic index (MI), mitotic phase frequencies, chromosome aberrations (CAs) and DNA damage in *Allium cepa* root cells were investigated with Allium ana-telophase and comet assay. Concentrations of 0.5xEC_{50} (25 ppm), EC_{50} (50 ppm) and 2xEC_{50} (100 ppm) of 2-CPY, Methyl methanesulfonate (MMS-10 ppm, positive control) and distilled water (negative control) were applied to *A. cepa* roots for 24, 48, 72 and 96 h. 2-CPY showed a cytotoxic effect by reducing root growth and MI, but also showed genotoxic effect by increasing CAs (disturbed ana-telophase, chromosome laggards, stickiness, bridges and polyploidy) and DNA damage at substantial levels. The amount of 2-CPY was shown to be increased statistically in both duration and dose by liquid chromatography-tandem mass spectrometry (LC-MS/MS). 2-CPY should be used carefully and investigated its cyto-genotoxic effects with other toxicology test systems.

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ÖZ

2-Kloropiridin (2-KP) kozmetiklerin, böcek ilaçlarının ve diğer farmasötik ürünlerin önemli bir öncüsüdür ve aynı zamanda endüstriyel atık sulardaki tarımsal kimyasal ürünlerin ve nehir kirleticilerin metabolitlerinin ürünleri olarak iz kimyasal olarak da tanımlanmaktadır. Bu çalışmada, *Allium cepa* kök hücrelerinde 2-KP'nin mitotik indeks (MI), mitotik faz frekansları, kromozom sapmaları (KA) ve DNA hasarı üzerine etkileri Allium ana-telofaz ve komet testi ile araştırılmıştır. *A. cepa* köklerine 0.5xEC_{50} (25 ppm), EC_{50} (50 ppm) ve 2xEC_{50} (100 ppm) 2-KP, Metil metansülfonat (MMS-10 ppm, pozitif kontrol) ve distile su (negatif kontrol) konsantrasyonları *A. cepa* köklerine 24, 48, 72 ve 96 saat boyunca uygulandı. 2-KP, kök büyümesini ve MI'yi azaltarak sitotoksik bir etki göstermişken KA'ları (bozulmuş ana-telofaz, kromozom kalgınları, yapışkanlık, köprü ve poliploidi) ve DNA hasarını önemli seviyelerde arttırarak genotoksik etki gösterdi. Sıvı kromatografi tandem kütle spektrometresi (LC-MS/MS) ile 2-KP miktarının hem süre hem de doza bağlı istatistiksel olarak arttığı gösterilmiştir. 2-KP dikkatli kullanılmalı ve sito-genotoksik etkilerini diğer toksikoloji test sistemleri ile araştırmalıdır.

1. Introduction

2-CPY is a non-naturally occurring environmental contaminant. It is the intermediate of pesticides such as imidacloprid, cosmetics and other pharmaceutical products such as pyrithione-based biocides. It is also used as a starting material in the production of antihistamine drug, pheniramine and the antiarrythmic disopyramide. It has been identified as a trace organic element in rocess streams and wastewater and a river pollutant (Goe 1982; Melcher and Bouyoucos 1990; Guardiola et al. 1991; Hendricks et al. 1994; Vlastos et al. 2010; Skoutelis et al. 2017). Although it is widespread in so many areas of use, there are very few studies on 2-CPY toxicity. Some properties about 2-CPY are given Table 1.

Table 1. Some properties of 2-CPY.

Chemical name	Synonyms	Chemical structure	Molecular Weight	Water solubility at 25°C
2-Chloropyridine	α-chloropyridine, o- Chloropyridine, 2-chloro-(9Cl)		113.544 g mol^{-1}	2.5 g 100 g^{-1}

Allium test has been used to assess the cytogenotoxic effects of environmental contaminants. Because the onions are easy to store and use, and the root tip cells form a suitable system for macroscopic (growth, EC_{50}) and microscopic parameters (MI and CAs like c-mitosis, micronucleus and stickiness etc. due to its large size and small number of chromosomes). It is also validated by United Nations Environmental Program, World Health Organization, and United States Environmental Protection Agency. Additionally, Allium test results show a good correlation with the other eukaryotic and prokaryotic test results (Grant 1982; Fiskesjö 1985; Rank and Nielsen 1994; Teixeira et al. 2003; Ma et al. 2005; Leme and Marin-Morales 2009; Kwasniewska et al. 2012; Rodriguez-Ruiz et al. 2014; Liman et al. 2015; Palmieri et al. 2016; Küçük and Liman 2018; Verma and Srivastava 2018).

The comet assay or single-cell gel electrophoresis in *A. cepa* root meristematic cells has been used for assessing of DNA damage of environmental contaminants. This assay is relatively low cost, simple, fast and reliable. It gives reproducible results and can be studied independent than mitosis and small number of cells (Seth et al. 2008; Türkoğlu 2012; Ventura et al. 2013; Jiang et al. 2014; Ciğerci et al. 2015; Silveira et al. 2017; Cortés-Eslava et al. 2018).

The purpose of this study was to assess the effects of 2-CPY on growth, MI, CAs and DNA damage in the root tips in *A. cepa* by Allium ana-telophase and comet assays. The estimation of total 2-CPY in *A. cepa* root tips was also analyzed using LC–MS/MS.

2. Materials and Methods

2.1. Materials

Equal-sized *A. cepa* L. bulbs (25–30 mm in diameter, without any cure) were purchased from local market in Uşak, Turkey. 2-CPY (99%, CAS Number 109-09-1), MMS (CAS No.67-27-3), basic fuchsine, disodium hydrogen phosphate, low melting point agarose (LMPA), ethidium bromide (EtBr), normal melting point agarose (NMPA), glacial acetic acid, hydrochloric acid, magnesium chloride hexahydrate, potassium chloride, potassium phosphate monobasic, sodium chloride, sodium hydroxide, Triton X-100, trizma hydrochloride, trizma base and EDTA were bought from Sigma Aldrich (Munich, Germany).

2.2. Growth inhibition test

The growth inhibition test was performed to determine the doses to be used to study the cyto-genotoxic effects of 2-CPY on *A. cepa* root meristem cells as described previously with some modifications (Liman et al. 2011). Different concentrations of 2-CPY (5, 10, 25, 50, 100, 200 and 400 ppm) and distilled water (control group) were applied to onions at room temperature (21 ± 4°C) for 96 h in the dark (Figure 1). Five onions were used for each application. In the end, average root length of the onions (ten roots from each onion) was measured according to Fiskesjö (1988). The effective concentration (EC_{50}) was determined as the dose that reduces the growth of root tips by 50% compared to the negative control group.

2.3. A. cepa ana-telophase test

A. cepa ana-telophase test was performed according to Rank and Nielsen (1994) with slight modifications. Onion bulbs were kept in the distilled water for two days to reach a length of 2-3 cm at room temperature (21 ± 4°C) in the dark. Roots were exposed to ½xEC_{50} (25 ppm), EC_{50} (50 ppm) and 2xEC_{50} (100 ppm) concentrations of 2-CPY along with negative (distilled water) and positive control (MMS, 10 ppm) for 24, 48, 72 and 96 h. Three onions were used for each application. 15-20 root tips about 1 cm long were fixed with Carnoy fixative (1:3 glacial acetic acid/ethanol, *v*/*v*) at 4°C for 24 h.

Figure 1. EC_{50} determination of 2-CPY on *A. cepa* root meristematic cells. Doses from left to right 0, 5, 10, 25, 50, 100, 200 and 400 ppm.

After washing distilled water, samples were taken into 70% alcohol for examination and stored at 4°C. 1-2 mL of 1N HCl solution was added to the root tips and kept in at 60°C for 8-10 min. Roots were stained with Feulgen method at room temperature for 25-30 min after washing with distilled water thrice with 5 min intervals. The dark stained root tips were cut with a razor blade, then one drop of 45% acetic acid was added to lysed it. The slides were prepared for MI and CAs by covering the coverslips with nail polish and evaluated by using Nikon Eclipse Ci-L light microscope (Japan), equipped with a CMOS camera (Argenit, Kameram5, Turkey).

For each application, MI values were determined by dividing mitosis cells into total cells and expressed as a percentage by counting 5000-5200 cells in five different roots. To determine CAs, 100 ana-telophase cells per slide (totally 500) were counted for each concentration and expressed as a percentage (Saxena et al. 2005). CAs in ana-telophase cells were recorded as disturbed ana-telophase, chromosome laggards, stickiness, anaphase bridge and polyploidy.

2.4. Comet assay

Seven root tips (~1 cm) were cut for each experiment and chopped with 600 µl precooled Tris-$MgCl_2$ buffer (0.5% w/v Triton X-100, 4 mM $MgCl_2$-$6H_2O$, 0.2 M Tris, pH 7.5). Samples were centrifuged at 1200 rpm for 7 minutes at 4ºC. While the pellet was used to determine DNA damage, the supernatant was used for quantitative analyses. Other steps of comet assay were carried out as reported earlier (Liman et al. 2019).

2.5. Determination of 2-CPY in A. cepa root meristem cells

LC-MS/MS (An Agilent 1200 series UPLC equipped with a binary pump system, cooling system, column oven and an auto-sampler) was used for the estimation of total 2-CPY in *A. cepa* root tips. Mass spectrometric detection was performed on an equipped with 6460 Triple Quadrapole LC–MS/MS system. The triple quadrupole mass spectrometer was equipped with electrospray jet stream ionization source system. Ion source is in positive electrospray ionization mode. Multiple reactions monitoring mode were used to operate the MS/MS system. For chromatographic separation of analysts, Zorbax Eclipse plus C18 (2.1×50 mm, 1.8 µ) column was used. The mobile phase was consisted of 0.1% formic acid in water 70% (mobile phase A) and acetonitrile 30% (mobile phase B), at total flow rate of 1.2 ml min^{-1} with a column temperature of 40°C. The chromatographic separation was achieved using 3.129 min isocratic elution. The following MRM transitions were monitored: m/z 114 → 77.9 and 114 → 51.1

2.6. Statistical analysis

The data were stated as the mean ± standard deviation. The comparison of the group averages was determined by using IBM SPSS Statistics for Windows, version 23 through the One-Way Analysis of Variance (ANOVA) and Duncan multiple range tests 5%. Pearson correlation test was used to determine the dose response and time response relationship.

3. Results and Discussion

The cyto-genotoxic effects of 2-CPY to *A. cepa* root meristematic cells were determined by Allium and comet assay. Table 2 shows the results of growth inhibition test of 2-CPY on *A. cepa* roots.

Table 2. Effect of 2-CPY on *A. cepa* root lengt.

Doses (ppm)	Average length (cm) ± SD*	Growth (%)	Decrease (-) in growth (%)
Control	3.81±0.21a	100.00	0
5	2.97±0.16b	77.95	22.05
10	2.72±0.16c	71.39	28.61
25	2.38±0.10d	62.47	37.53
50	1.94±0.12e	50.92	49.08
100	1.35±0.10f	35.43	64.57
200	0.89±0.11g	23.36	76.64
400	0.37±0.10h	12.46	87.54

*Means with the same letter do not differ statistically at the level of 0.05. SD: Standard deviation.

The EC_{50} of 2-CPY was found to be 50 ppm (50.92%). All concentrations of 2-CPY decreased the mean root elongation statistically, with a dose-dependently (r= -0.975 p= 0.01). The applied doses of 2-CPY reduced the mean root length by 22.05% (5 ppm) to 87.54% (400 ppm). Inhibition of root growth could be not only related to apical meristematic activity (Webster and Macleod 1996) but also cell elongation during differentiation (Fusconi et al. 2006) or enzyme activation that promote the elongation and loosening of the cell wall in the differentiation process (Silveira et al. 2017). 15 min EC_{50} of 2-CPY was found as 1.64 mmol l^{-1} by the Microtox bacterial assay (Wu and Huang 1998). The LD_{50} of 2-CPY in rabbits after skin application or intraperitoneal injection was found 64 and 48 mg kg^{-1}, respectively (Gehring et al. 1967). LD_{50} of 2-CPY in mouse after oral application was found 110 mg kg^{-1} (Shimizu et al. 2000). IC_{50} of 2-CPY derivatives possessing 1,3,4-oxadiazole moiety was found to vary between 1.61±0.06 and >20 µg ml^{-1} in gastric cancer cell SGC-7901 (Zheng et al. 2010).

The effect of 2-CPY on MI and mitotic phases in *A. cepa* in root meristematic cells is shown in Table 3. All doses of 2-CPY were significantly reduced to MI. The decreased MI for 2-CPY was found statistically significant not only dose dependently for 24 h (r= -0.968 p= 0.01), for 48 h (r= -0.974 p= 0.01), for 72 h (r= -0.933 p= 0.01) and for 96 h (r= -0.944 p= 0.01) but also time dependently for 25 ppm (r= -0.989 p= 0.01), for 50 ppm (r= -0.974 p= 0.01) and for 100 ppm (r= -0.949 p= 0.01). This inhibition of MI suggests that 2-CPY has a potentially cytotoxic effect. MI values at 100 ppm were found to be lower than MMS, but the decrease in 48 h was only statistically significant. Significant reduction in MI may be due to disturbed cell cycle such as blockage of G_1 phase and suppressing DNA synthesis or inhibition of DNA synthesis at the S phase (Sudhakar et al. 2001; Gupta et al. 2018) or blocking of G_2 phase preventing the cell from entering mitosis (El-Ghamery et al. 2000), or mitotic phase duration changes (Chauhan and Gupta 2005). It also may due to inhibition of specific proteins of cell cycle remains as possible target site which inhibit DNA polymerase and other enzymes resulting in antimitotic effect (Hidalgo et al. 1989) or and ROS disturbance homeostasis (Livanos et al. 2012). Unlike our result, 2-CPY did not show any cytotoxic effect in human lymphocytes (Stapleton et al. 2008). While 2-CPY decreased the prophase index statistically (except at 100 ppm 48, 72 and 96 h), it increased the telophase index. The reduction of the prophase index may be due to the blockage of interphase cells of the transition of the prophase (Soliman and Ghoneam 2004). The increasing of telophase index may be due to delay in mitotic cycle accomplishment (Rangaswamy et al. 1981).

2-CPY significantly induced total CAs (disturbed ana-telophase, chromosome laggards, stickiness, anaphase bridge and polyploidy) in *A. cepa* ana-telophase cells (Table 4 and Figure 2). The concentration (for 24 h p= 0.829 r= 0.01, for 48 h p= 0.89 r= 0.01, for 72 h p= 0.791 r= 0.01, and for 96 h p= 0.882 r= 0.01), time dependent (for 25 ppm r= 0.979 p= 0.01, for 50 ppm r= 0.934 p= 0.01, and for 100 ppm r= 0.929 p= 0.01) increase of total CAs described genotoxic potentiality of 2-CPY. But these total CAs were lower than MMS. Chromosome laggards (4% at 100 ppm for 96 h) were the most common CAs, while polyploidy (0.4% at 25 and 100 ppm for 24 h) was the least common CAs. Disturbed ana-telophase and chromosome laggards may result from deformation of the spindle structure or degraded microtubules (Evseeva et al. 2005; Kumari et al. 2009; Singh and Roy 2017). Chromosome laggards may also cause micronucleus formation (Leme and Marin-Morales 2009). Stickiness which leads to DNA-DNA or DNA-protein crosslinking may possibly be caused by the

Table 3. Effect of 2-CPY on mitotic and phase index in *A. cepa* root meristematic cells.

Concentration (ppm)	*CCN*	MI±SD*	Phase index (%)±SD*			
			Prophase	Metaphase	Anaphase	Telophase
Control-24 h	5084	71.54±0.57a	89.30±0.46a	1.90±0.12a	2.37±0.21a	6.43±0.26a
MMS-10	5119	56.83±0.76b	88.04±0.61b	2.86±0.27b	2.58±0.26ab	6.53±0.31a
25	5123	62.15±0.29c	85.39±0.31c	2.54±0.19c	2.67±0.23b	9.39±0.21b
50	5123	58.6±0.35d	84.65±0.37d	2.56±0.23c	2.7±0.14b	10.09±0.21c
100	5081	56.84±0.55b	85.49±0.24c	2.49±0.18c	2.84±0.16b	9.18±0.24b
Control-48 h	5114	70.91±0.51a	88.58±0.24ab	2.40±0.14a	2.65±0.19a	6.37±0.23a
MMS-10	5094	54.26±0.66b	88.25±0.36bc	2.37±0.16ab	2.84±0.13a	6.54±0.28a
25	5076	58.02±0.60c	86.45±0.21d	2.21±0.17bc	2.72±0.17a	8.62±0.16b
50	5107	54.59±0.64b	87.90±0.34c	2.09±0.08c	2.31±0.14b	7.70±0.19c
100	5084	51.6±0.77d	88.83±0.26a	1.82±0.11d	2.01±0.11c	7.33±0.18d
Control-72 h	5098	69.50±0.70a	88.94±0.31a	2.12±0.09a	2.43±0.11a	6.52±0.25a
MMS-10	5085	50.58±0.42b	88.76±0.28a	2.10±0.15a	2.76±0.15b	6.38±0.33a
25	5177	54.27±0.92c	87.94±0.22b	2.09±0.16a	2.38±0.1b	7.59±0.36b
50	5092	50.37±0.83b	88.09±0.13b	2.01±0.09a	2.29±0.21bc	7.62±0.16b
100	5105	48.57±0.77d	88.67±0.40a	1.98±0.17a	2.1±0.2c	7.26±0.33b
Control- 96 h	5074	70.77±0.59a	89.00±0.38a	2.23±0.12a	2.53±0.16ab	6.24±0.24a
MMS-10	5083	47.5±0.79b	88.07±0.1b	2.03±0.18ab	2.57±0.2a	7.33±0.31b
25	5087	51.17±0.57c	88.32±0.54bc	1.88±0.18b	2.38±0.2ab	7.41±0.39b
50	5094	48.78±0.53d	88.25±0.28bc	1.97±0.19b	2.25±0.18bc	7.52±0.34b
100	5089	47.35±0.57b	88.74±0.31ac	1.89±0.17b	2.10±0.25c	7.28±0.18b

*Means with the same letter in the same column for each application time do not differ statistically at the level of 0.05. Cell Numbers SD: Standard Deviation.

Table 4. Chromosome aberrations observed on *A. cepa* ana-telophase cells exposed to 2-CPY.

Concentration (ppm)	CCN	Anaphase-Telophase Anomalies %					
		DAT	CL	S	AB	P	TA± SD*
Control-24 h	500	1	0.6	0.8	0.6	0.6	3.60±0.55a
MMS-10 ppm	500	2.4	3.2	2.8	3.4	2	13.8±0.84b
25	500	2.6	2.8	2.6	0.8	0.4	9.20±0.45c
50	500	2.8	2.6	3	1.4	0.8	10.60±0.55d
100	500	2	3.4	3.4	2.2	0.4	11.40±0.89d
Control-48 h	500	0.6	1	0.6	0.8	0.2	3.20±0.45a
MMS-10 ppm	500	2.8	3	3.2	3.2	2.2	14.4±0.89b
25	500	2.6	2.6	2.6	1.8	0.8	10.40±0.55c
50	500	2.8	2.6	2.6	2.4	1.2	11.60±0.55d
100	500	2.6	3	2.8	3	1.6	13.00±0.71e
Control-72 h	500	1.4	1.2	0.6	0.8	0.2	4.20±0.45a
MMS-10 ppm	500	3	3.2	4	3.2	2.6	16.00±0.71b
25	500	2.8	3	3.2	2.4	1.2	12.60±0.55c
50	500	3	2.8	3.2	3.2	1.4	13.60±0.89d
100	500	3.2	3.2	3.4	3.2	1.8	14.8±0.84e
Control-96 h	500	0.6	1.2	0.4	1	0.2	3.40±0.55a
MMS-10 ppm	500	3.6	3.4	4.2	3.6	2.4	17.20±0.45b
25	500	2.4	3.2	3.6	2.6	1.6	13.40±0.55c
50	500	3.4	3.4	3.6	3.2	1.4	15.00±0.71d
100	500	3.4	4	3.8	3.6	1.4	16.4±0.89b

* Means with the same letter in the same column for each application time do not differ statistically at the level of 0.05. SD: Standard Deviation. CCN: Counting Cell Numbers. DAT: Disturbed Anaphase-Telophase. CL: Chromosome Laggards. S: Stickiness. AB: Anaphase Bridge. P: Polyploidy. TA: Total Anomalies.

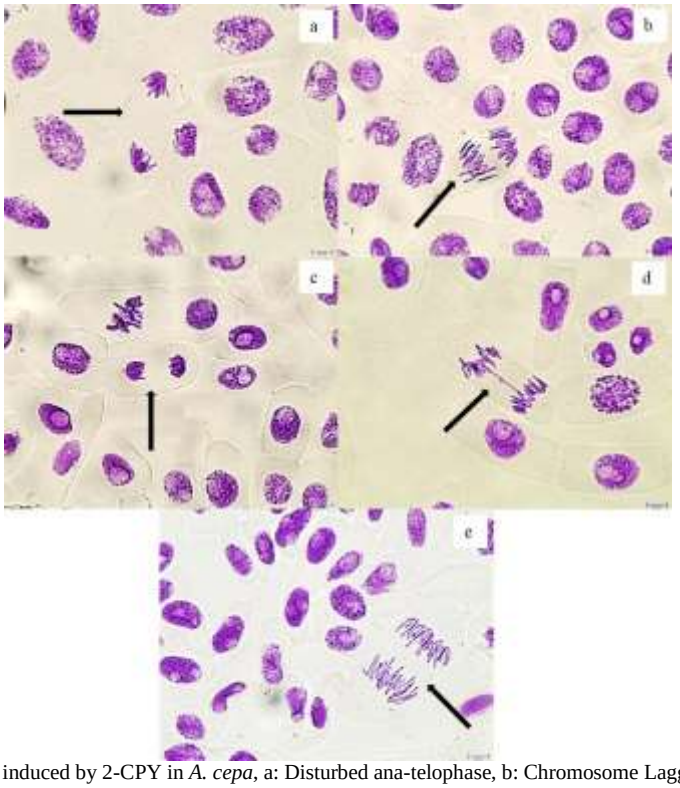

Figure 2. Ana-telophase anomalies induced by 2-CPY in *A. cepa*, a: Disturbed ana-telophase, b: Chromosome Laggards, c: Stickiness, d: Anaphase Bridge, e: Polyploidy.

depolymerization of DNA or partial dissolution of the nucleoproteins or the chromosomal intercalation of the chromatin fibers (Fiskesjö and Levan 1993; Türkoğlu 2015; El-Ghamery and Mousa 2017). Anaphase bridge may occur due to the clastogenic effect of the chemicals such as breakage or fusion of chromosomes, unequal chromatid exchange, dicentric chromosome formation, alteration of the activation of replication enzymes or incomplete adhesion (El-Ghamery et al. 2000; Luo et al. 2004; Bonciu et al. 2018). Polyploidy may occur the cytokinesis process disruption which leads to difficulties in the fragmoplast formation (Fernandes et al. 2007). 2-CPY induced mitotic aneuploidy in *Saccharomyces cerevisiae* (Zimmermann et al. 1986). 2-CPY showed clastogenic effect in the L5178Y mouse lymphoma cells by inducing chromosome aberrations and micronuclei formation with or without metabolic activation (Dearfield et al. 1993). Micronucleus formation was statistically increased in human lymphocyte cultures treated *in vitro* with 2-CPY at 100 µg ml^{-1} but it was not found genotoxic at or below 50 µg ml^{-1}. Photo-treatment of 2-CPY also produced genotoxic products at 100 µg ml^{-1} (Vlastos et al. 2010). But, 2-CPY did not show any cytotoxic and clastogenic effects in African Green monkey kidney cell lines in the dose range from 400 to 3200 µg ml^{-1} (Anuszewska and Koziorowska 1995). Frequencies of micronucleated normochromatic erythrocytes did not change in mice after exposed to 2-CPY (10 to 1000 ppm) in drinking water for 3 months (Roberts 2017).

The comet assay results are shown in Table 5. 2-CPY induced DNA damage not only dose dependently (for 24 h p= 0.938 r= 0.01, for 48 h p= 0.89 r= 0.01, for 72 h p= 0.897 r= 0.01, and for 96 h p= 0.977 r= 0.01) but also time dependently (for 25 ppm r= 0.85 p= 0.01, for 50 ppm r= 0.896 p= 0.01, and for 100 ppm r= 0.934 p= 0.01). The DNA damage caused by 2-CPY ranged from 100.67±3.06 to 148.67±1.57. But these values were lower than MMS. 2-CPY was found to be mutagenic in Ames test system with metabolic activation (Claxton et al. 1987; Roberts 2017). The reactive oxygen species (ROS) produced in *Salmonella typhimurium* treated with 2-CPY may cause DNA damage and CAs (Chlopkiewicz et al. 1993).

Quantitative analysis of 2-CPY in *A. cepa* roots by LC-MS/MS is shown Table 6. The amount of 2-CPY was increased depending on both duration (for 25 ppm r= 0.923 p= 0.01, for 50 ppm r= 0.908 p= 0.01 and for 100 ppm r= 0.948 p= 0.001) and dose (for 24 h r= 0.886 p= 0.01, for 48 h r= 0.977 p= 0.01, for 72 h r= 0.984 p= 0.01 and for 96 h r= 0.984 p= 0.01). The lowest amount of 2-CPY was obtained at 25 ppm 24 h (0.109±0.006 ppb), while the highest amount of 2-CPY was obtained at 100 ppm in 96 h (0.109±0.006 ppb).

Table 5. DNA damage induced by 2-CPY on *A. cepa* roots.

Compounds	Concentration (ppm)	DNA Damage (Arbitrary Unit ±SD)*			
		24 h	48h	72 h	96 h
Negative control	-	4.33±0.58a	3.67±0.58a	6.33±0.58a	4.67±1.15a
MMS	10	146.33±2.31b	151.33±1.53b	155±1.73b	158.67±2.52b
	25	100.67±3.06c	127±2.65c	129.67±2.08c	132.33±1.15c
2-Chloropyridine	50	107.33±2.08d	130.67±1.53c	135.67±3.21d	139.33±2.08d
	100	130.67±2.89e	137.67±3.21d	140±2e	148.67±1.53e

* Means with the same letter in the same column do not differ statistically at the level of 0.05. SD: Standard Deviation.

Table 6. The amount of 2-CPY in *A. cepa* root meristem cells.

Compounds	Concentration (ppm)	Amount (ppb ±SD)*			
		24 h	48h	72 h	96 h
Negative control	-	-	-	-	-
MMS	10	-	-	-	-
2-Chloropyridine	25	0.109±0.006a	0.138±0.005a	0.148±0.007a	0.164±0.01a
	50	0.121±0.005b	0.187±0.004b	0.196±0.008b	0.215±0.006b
	100	0.129±0.002c	0.227±0.013c	0.253±0.009c	0.291±0.007c

*Means with the same letter in the same columns do not differ statistically at the level of 0.05; ppb: parts per billion; SD: Standard Deviation.

4. Conclusion

2-CPY induced cytotoxicity by reduction in root growth and MI, and genotoxicity by increasing CAs and DNA damage to *A. cepa* roots. For this reason, it is necessary to be careful when using 2-CPY and necessary to carry out further molecular toxicological experiments included in the cyto-genotoxicity of 2-CPY on plants.

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