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The Evaluation of the Genotoxic and Cytotoxic Effects of Pyriproxyfen Insecticide on *Allium cepa* Somatic Chromosomes with Mitotic Activity, Chromosome Abnormality and Micronucleus Frequency

Pyriproxyfen İnsektisitinin *Allium cepa* Somatik Kromozomları Üzerinde Mitotik Aktivite, Kromozom Anormallikleri ve Mikroçekirdek Sıklığıyla Genotoksik ve Sitotoksik Etkilerinin Değerlendirilmesi

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Abstract

In this study, the genotoxic and cytotoxic effects of pyriproxyfen insecticide was evaluated by investigating mitotic index and phase, chromosomal abnormalities, and micronucleus proportion on root tip cells of *Allium cepa* L. The root types were applied to 0.25, 0.5, 1 and 2 ppm test solutions within 12, 24 and 36 h. Mitotic index was markedly lessened with increasing pyriproxyfen in each treatment group as compared to the controls. The percentages of mitotic phases have been meaningfully impacted. Pyriproxyfen noticeably heightened the anomaly cell ratio in most of the utilized solutions and application periods when compare to control. Mitotic abnormalities were determined as sticky, disturbed prophase, c-mitosis, chromatid bridges and laggards. Furthermore, micronucleus frequency was calculated at interphase.

Keywords: *Allium* test, chromosome, cytotoxicity, genotoxicity, micronucleus, Pyriproxyfen

Öz

Bu çalışmada, pyriproxyfen insektisitinin genotoksik ve sitotoksik etkileri *Allium cepa*'nın kök hücreleri üzerinde mitotic indeks ve faz, kromozomal anormallikler ve mikroçekirdek oranının araştırılmasıyla değerlendirildi. Kök örneklerine 0.25, 0.5, 1 ve 2 ppm test solüsyonları 12, 24 ve 36 saat uygulandı. Mitotik indeks her test grubunda artan pyriproxyfen konsantrasyonlarıyla kontroller ile karşılaştırıldığında açık bir şekilde düşürüldü. Mitotik faz oranları önemli bir şekilde etkilendi. Pyriproxyfen dikkate değer seviyede kullanılan test solusyonlarında ve uygulama periyotlarında kontrol grupları ile karşılaştırıldığında anormal hücre oranını arttırdı. Mitotik anormallikler yapışık kromozom, düzensiz profaz, c-mitoz, kromatid köprüleri ve geri kalan kromozom olarak belirlendi. Ayrıca, mikroçekirdek sıklığı interfazda hesaplandı.

Anahtar Kelimeler: *Allium* test, genotoksisite, kromozom, mikroçekirdek, pyriproxyfen, sitotoksisite

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1. Introduction

Pesticides are generally utilized in agricultural fields since prevent yield losses outcoming from different influences like pests (Karaismailoglu et al. 2013; Karaismailoglu 2015). They and derivatives polluted with them contain persistently the harms on life systems with dangers of teratogenicity (Liman et al. 2011).

Insecticides are applied to control insects in agriculture. Pyriproxyfen is a dominant insecticide (Ishaaya and Horowitz 1995). It is a pyridine-based pesticide which simply melts in water, and causes problems in living systems (EPA 1999; Coskun et al. 2015). It is used in Turkey to control pests such as *Bemisia tabaci* Gennadius*, Aonidiella aurantii* Maskell*, Pseudaulacaspis pentagona* Tozzetti in cotton, eggplants, citrus, peaches, greenpeppers and tomatoes agricultural areas at 0.5 ppm concentrations (MARA 2009).

Plants have helpful effects in following toxic impacts in life systems of the chemical as pesticides (Karaismailoglu 2015) with examining mitotic index, chromosomal abnormalities, and micronucleus frequency on the root tip cells. The most extensively utilized plants are *Allium cepa* (Turkoglu 2009; Karaismailoglu 2015), *Vicia* faba (Khadra et al. 2012) and *Helianthus annuus* (Karaismailoglu et al. 2013). Particularly, *A. cepa* is one of the primarily used plant in genotoxicity tests meanwhile this plant is highly vulnerable and reliable among various tests (Grant 1994; Karaismailoglu 2015).

The aim of this study was to define the impacts of the pyriproxyfen insecticide on mitotic index and phase, chromosomal abnormalities, and micronucleus frequency on the root tip cells of *Allium cepa* L.

2. Materials and Methods

Allium cepa (2n=16) were obtained from a commercial shop. Pyriproxyfen [4-phenoxyphenyl (RS)-2-(2-pyridyloxy) propyl ether] (Ishaaya and Horowitz 1995; Coskun et al. 2015) was purchased from commercial market and used as stock solution in toxicity tests.

When root lengths of onions lengthened 1 cm length, they were treated with 0.25, 0.5, 1, and 2 ppm pyriproxyfen solutions, at 12, 24 and 36 h treatment times. The treatment concentrations were noticed as grounded on the dose of pyriproxyfen in accordance with the used dose in agriculture field, and its multiples (MARA 2009). Distilled water was used as control. Tips were fixed in 3 ethanol- 1 glacial acetic acid and deposited at 4°C overnight. Later, they were hydrolyzed in 1 N HCl at 60°C for 10 min and stained by Schiff's reagent for 1.5 h. Five slides were selected randomly from each application group for the genotoxic and cytotoxic examination and mitotic index (MI), micronucleus (MN) in interphase, and chromosome aberrations such as sticky, disturbed prophase, c-mitosis, chromatid bridges and laggards were examined in the dividing cells (Karaismailoglu 2014a; 2014b).

The data are performed by utilizing analysis of variance (ANOVA) with SPSS (2008) computer program and level of significance is determined as $p<0.05$. Dunnett multiple range test was used for the determination of statistical significance among the monitored variations. The results of statistical examinations are given in Tables 1-3.

3. Results

During The effects of utilized concentrations of pyriproxyfen insecticide on MI and the percentages of mitotic stages are given in Table 1. Commonly, utilized doses of pyriproxyfen noticeably diminished MI of bulb root tip chromosomes in related to the control ($p<0.05$). Except as, MI doesn't show significant difference in 0.25 ppm of pyriproxyfen at all application times as compared to the control groups. There were markedly differences in MI of onion somatic cells applying doses of pyriproxyfen in comparison with the control (Table 2). Because of 2 ppm pyriproxyfen treatment, MI considerably deteriorate in each application time (Table 1). Besides, when mitotic stage percentages were related with the control in treatment times, there were statistically significant outcomes as well ($p<0.05$). Almost all the applications significantly impacted the mitotic stage percentages (Table 1). Also, a significant rise in percentages of abnormalities in stages with growing pyriproxyfen concentrations was defined (Table 1).

Table 1. Effects of the treated pyriproxyfen doses and control on mitotic cell division in *A. cepa*

Time	Concentrations	Number of	MI±SD	Prophase (%)		Metaphase (%)		Ano-telophase (%)	
(h)	(ppm)	examined cells		T	AN	T	AN	T	AN
	C	5000	16.75±0.24	39.48	-	34.13	-	26.39	-
	0.25	5000	16.66±0.15	34.41*	-	28.45*	-	37.14*	-
12	0.5	5000	14.99±0.19*	35.46*	2.05*	29.88*	0.98*	34.66*	0.09*
	1	5000	14.23±0.28*	29.66*	1.72*	28.99*	2.10*	41.65*	2.15*
	2	5000	12.05±0.12*	44.61*	2.90*	35.77*	1.95*	19.62*	1.77*
	C	5000	18.25±0.18	37.61	-	34.02	-	28.37	-
	0.25	5000	18.02±0.25	31.44*	-	34.11	-	34.45*	-
24	0.5	5000	14.91±0.37*	25.99*	1.59*	34.10	1.85*	39.91*	1.63*
	1	5000	13.88±0.22*	26.99*	2.05*	30.75*	1.88*	42.26*	2.01*
	2	5000	11.44±0.28*	24.56*	3.01*	33.04*	2.72*	42.40*	3.15*
	C	5000	19.77±0.21	41.51	-	38.16	-	20.33	-
	0.25	5000	19.65±0.47	40.75*	0.61*	37.75*	0.44*	21.50*	0.11*
36	0.5	5000	16.08±0.33*	43.21*	2.85*	39.98*	4.02*	16.81*	0.78*
	1	5000	12.45±0.41*	41.53	3.48*	37.01*	2.55*	21.46*	2.91*
	2	5000	10.62±0.27*	36.77*	4.49*	38.15	5.11*	25.08*	5.22*

*Different from the control $p<0.05$, MI: mitotic index; SD: standard deviation; C: control, T: total, AN: abnormal.

Table 2. Chromosomal abnormalities in the root tips of *A. cepa* exposed to concentrations of pyriproxyfen and control

Time	Concentrations	Abnormality percentages (%)					Total
(h)	(ppm)	Sticky	Disturbed prophase	Chromatid bridge	c-mitosis	laggards	Abnormality
	C	-	-	-	-	-	-
	0.25	-	-	-	-	-	-
12	0.5	0.44±0.10*	0.28±0.04*	0.11±0.04*	0.16±0.08*	0.22±0.06*	1.21*
	1	0.89±0.15*	0.44±0.10*	0.23±0.08*	0.35±0.06*	0.41±0.04*	2.32*
	2	1.91±0.21*	2.76±0.27*	1.98±0.41*	1.21±0.12*	1.08±0.10*	8.94*
	C	-	-	-	-	-	-
	0.25	-	-	-	-	-	-
24	0.5	0.85±0.11*	1.01±0.15*	0.67±0.11*	0.48±0.18*	0.98±0.21*	3.99*
	1	1.31±0.08*	2.74±0.33*	1.22±0.18*	1.45±0.11*	1.41±0.42*	8.13*
	2	4.11±0.24*	3.05±0.27*	0.88±0.12*	1.11±0.27*	1.68±0.25*	10.83*
	C	-	-	-	-	-	-
	0.25	0.22±0.06*	0.11±0.08*	-	0.33±0.12*	-	0.66*
36	0.5	0.44±0.04*	2.29±0.15*	1.79±0.11*	1.98±0.24*	1.43±0.18*	7.93*
	1	1.95±0.24*	2.04±0.33*	3.09±0.18*	1.04±0.10*	1.68±0.15*	9.80*
	2	3.25±0.41*	4.98±0.18*	4.56±0.36*	3.95±0.18*	3.51±0.33*	20.25*

*Different from the control p<0.05, C: Control.

Chromosome anomalies were examined in mitotic phases, and their types and frequencies are shown in Table 2 and Figure 1. Treatments triggered five types of abnormalities: sticky, disturbed prophase, c-mitosis, chromatid bridges and laggards.

Table 3. The effects of pyriproxyfen insecticide on the micronucleus assay

Time	Concentrations	MN
(h)	(ppm)	(%)
	C	-
	0.25	-
12	0.5	0.69±0.08*
	1	0.41±0.15*
	2	0.73±0.14*
	C	-
	0.25	-
24	0.5	0.16±0.06*
	1	0.54±0.15*
	2	0.96±0.10*
	C	-
	0.25	-
36	0.5	0.37±0.08*
	1	0.64±0.04*
	2	1.39±0.16*

*Different from the control p<0.05, C: Control, MN: Micronucleus.

The results of the micronucleus analysis in *A. cepa* somatic cells exposed to control group and doses of pyriproxyfen are given in Table 3 and Figure 1. MN frequency was markedly higher at 2 ppm than the other treatment of pyriproxyfen in all the application groups (Table 3). In addition, at 12, 24 and 36 h treatments of 0.5 and 1 concentrations of pyriproxyfen enhanced noticeably MN frequency.

4. Discussion

The uncontrolled practice of chemical agents such as pesticides has usually triggered to ecological contamination, resulting in reverse effects on life systems. Hence, assessment of the impacts of pyriproxyfen insecticide and its effects on mitotic index and chromosomes permit valuable data concerning the influences of these generally applicated genotoxic or cytotoxic things (Karaismailoglu 2015).

MI may be utilized as sign of cell increase, which analyzes the percentage of cells in different mitotic stages (Ping et al. 2012). The influences of various doses of pyriproxyfen on MI in *A. cepa* root tip cells are presented in Table 1. Mitotic influence mostly lessened with increase pyriproxyfen concentrations at each treatment times in comparison with control groups (p<0.05). Moreover, at doses of 0.25 ppm pyriproxyfen, MI was not significantly difference to control at almost all the application times (Table 1). Also, 2 ppm of pyriproxyfen was the most genotoxic and cytotoxic concentration, and has further mitodepressive outcome than 0.25, 0.5 and 1 ppm treatments at all application groups. If the MI percentage

decline below 22% by the side of control, this status persuades lethal effects on life systems. Moreover, the decreases under 50% have sublethal effects and mentioned the genotoxicity and cytotoxicity limit value (Panda and Sahu 1985; Karaismailoglu 2014b; 2015). In this investigation, sublethal impact was determined in 2 ppm as compared to control in 12, 24 and 36 h applications, and sublethal effect values were originated as 28.05%, 37.31% and 46.28%, respectively. These outcomes are suitable with results got from previous researches (Ping et al. 2012; Khadra et al. 2012; Karaismailoglu 2013; 2014a; 2015).

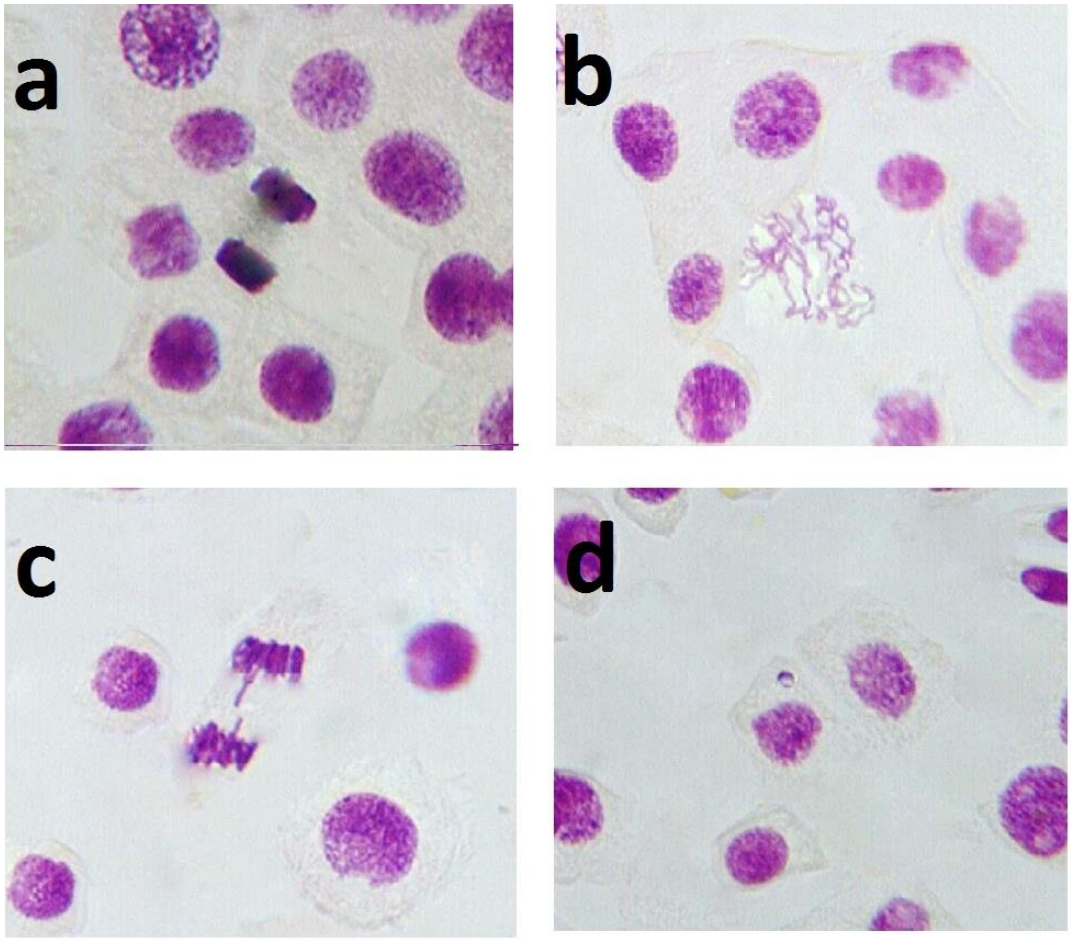

Figure 1. Chromosomal aberrations caused with pyriproxyfen in root tip cells of *A. cepa*; **a:** sticky, **b:** disturbed prophase, **c:** chromatid bridge **d:** micronucleus.

The effects of the different test concentrations on phases of the cell cycle in *A. cepa* root tip cells are presented in Table 1. When the phase frequencies are related with control in different treatment groups, there are statistically significant outcomes ($p<0.05$). The percentages of the mitotic phases were obviously influenced in completely almost all applications ($p<0.05$). When percentages of prophase and metaphase increased, ana-telophase reduced in observed cells. The effects on mitotic phases of pyriproxyfen solutions may be linked to barrier of prophase or detention in the mitotic phases in response to mitotic-stress (Liman et al. 2011).

Chromosome abnormalities can be used to observe of genotoxic and cytotoxic effects of the chemical agents like pesticides (Karaismailoglu 2015). The effects of pyriproxyfen concentrations and the control treatments on mitotic abnormalities and percentages of total abnormality in root tip cells are shown in Table 2. Perceived chromosomal abnormalities were determined as sticky, disturbed prophase, c-mitosis, chromatid bridges and laggards, and showed in Figure 1. The most commonly perceived type of chromosomal abnormality was sticky, which may result from disturbance in form of the genetic material (Mercykutty and Stephen 1980; Karaismailoglu 2014b). Also, disturbed prophase consists of outcoming of chromatin corrosion. Furthermore, c-mitosis constitutes with stopping spindle form such as colchicines influence (Badr 1983). Additional most common abnormality type chromatid bridges constitute with breaks of chromatins (Shehab and Adam 1983). Sticky, chromatid bridge and disturbed prophase have genotoxic and cytotoxic influences and permanent (Karaismailoglu 2015), and they can induce cellular losses. In addition, laggards are constituted with disappointment of spindle fiber construction, and it shows a low genotoxic and cytotoxic influence and may be recycle (Fiskesjö 1985).

Micronucleus analysis have important role in assessment of the genotoxicity and cytotoxicity impacts of pesticides (Gebel et al. 1997; Karaismailoglu 2013; 2014a; 2014b; 2015). MN creation and its rates in application times are given in Table 3 and Figure 1. MN percentage obviously enlarged with increasing pyriproxyfen concentrations as compared to the control groups, as an exception 0.25 ppm at all application times ($p<0.05$). MN rate was obviously more at the highest pyriproxyfen dose (2 ppm) than others.

As a result, expose to pyriproxyfen can present a genotoxic and cytotoxic threat to genetic material in *Allium cepa*. Whereas this insecticide arrests loss of crop and improve production in agricultural areas, it is not applied to hold beck it completely. However, this publication gives that if pyriproxyfen is used in test solutions below 0.5 ppm, the genotoxic and cytotoxic influences on *A. cepa* could be reduced.

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