Genotoxicity of Quizalofop-p-Ethyl on Vicia Faba Root Cells Micronucleus Induction and Mitotic Index

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ABSTRACT

Quizalofop-p-ethyl ($C_{19}H_{17}ClN_2O_4$-QPE) is a widely used herbicide for controlling annual and perennial grass weeds in broad-leaved agricultural crops. In this study, the effect of Quizalofob-P-Ethyl (QPE), which is used as a herbicide, on mitosis and micronuclei formation in Vicia faba root meristem cells was investigated. In this study, 5 different experimental sets as negative control (distilled water), positive control (cyclophosphomide-5mM) and three different doses of QPE (1.5mg/L, 3mg/L and 5mg/L) were used as the experimental group. Bean seeds of the same size were used as test material. QPE induced the MN formation in V. faba. It is observed that the increase in MN frequency and decrease in the MI are dose-dependent. There is a statistically significant difference between treatment and control groups ($P < 0.05$ and $p < 0.01$ for 3 mg/mL and 5 mg/ml respectively) for MN frequency. Results of our study indicate that QPE may produce genotoxic effects in plants. In addition, plant bioassays as the results of the majority of in vivo/in vitro mammalian tests are comparable, they are sensitive indicators for genotoxicity assessment.

Keywords: Quizalofop-p-ethyl ($C_{19}H_{17}ClN_2O_4$-QPE); Vicia faba; Micronucleus; mitotic index; genotoxicity; cytotoxicity.

1. INTRODUCTION

Agricultural ecosystems are, in a sense, artificialized ecosystems with many additional energy contributions such as fertilizers and pesticides in various forms due to the efforts of people to increase production, unlike natural ecosystems. Pesticides, substances used to control and prevent unwanted plants and living things are substances used as plant growth regulators, defoliants and dehumidifiers. Different pesticides and plant growth regulators are widely used in modern agriculture; Although the use of these chemicals has become a necessity, their frequent and indiscriminate use has many undesirable consequences for crop plants (Ehsan et al., 2016).

The amount of pesticide applications reaching the target organism is between 0.015-6%. The remaining 94-99.9% reaches non-target organisms and soil in the agroecosystem or mixes with the waters as chemical pollutants due to drifting and flowing into the surrounding natural ecosystems. Pesticides, that is, agricultural poisons, used in agriculture to combat weeds and other disease factors such as insects and fungi that can harm the product, damage our health and natural assets, while on the other hand, these factors that damage the product gain resistance and destroy the vitality of the soil and impoverish it (Carvalho, 2017).

From an agricultural point of view, herbicides also cause toxicity in crop plants. Applying herbicides above the dose and number of applications to be applied, applying with unsuitable herbicide mixtures and unsuitable environmental conditions cause toxicity in cultivated plants. Herbicide toxicity can occur in the leaves, stems, flowers and fruits of the plants, the plant remains weak and thus the crop plant is vulnerable to disease factors, pests and adverse environmental conditions, and yield losses increase even more (Greenland, 2003; Charles, 2013; Derr, 2016).

While herbicides are very important to agriculture, under certain circumstances they may act as pollutants that can deteriorate soils, ground waters and surface waters. It has been reported that the herbicide Quizalofop-p-Ethyl causes morphological changes in sunflower roots, and a decrease in mitotic index and an increase in mitotic irregularities depending on the concentration increase. (Karaismailoğlu ve ark., 2013; Mahakavi ve ark 2014; Yıldız and Arıkan, 2007).

The mitotic index is the ratio of mitotic cells to all cells in a population of cells. The mitotic index is an acceptable cytotoxic criterion for all living organisms. If the reduction in the mitotic index rate exceeds 50%, it may have lethal effect on the test organism. Generally, cytotoxic substances show their effects on mitosis by inhibiting microtubule formation (Heddle, 1991).

Since the 1900s, many genotoxicity tests have been developed to measure the carcinogenic potential of genotoxic substances. Significant progress has been made recently in the development of short-term test systems for the detection of environmental mutagens. In vitro tests include many tests that are more convenient, economical, and faster than in vivo tests to detect mutations, chromosome breaks, and other genetic effects (Barile, 2008). Micronuclei are defined as formations that arise during the mitotic division of the cell, are not included in the main nucleus, and originate from whole chromosomes or acentric chromosome fragments. These formations result from gene deficiencies, defects in the mitotic spindle, kinetochore or other parts of the mitotic apparatus, and chromosomal damage. (Cotello, 1999; Fenech, 1999). In recent years, it has also used for the detection the clastogenicity of environmental mutagens by many researhers (Sunyayar et al, 2006; Jha and Singh, 1994; Ma et al., 1995). Micronucleus (MN) method is considered the most convenient and fastest (Çelik et al., 2003; Stopper and Müller 1997)

The aim of this study was to evaluate the genotoxic effect of Quizalofop-p-Ethyl herbicide on Vicia faba by mitotic index and micronucleus test.

2. MATERIAL AND METHOD

2.1. Treatment solution and root tip preparations

Bean seeds of the same size were used as test material. For the V.faba test, dry broad bean seeds were soaked for 24 h in ultradistilled water and allowed to germinate between two layers of moist cotton at 24 ^{0}C. When the newly emerged roots were of 1.00–2.00 cm in length 10 seedlings for each treated group were selected seedlings were transferred into container containing Quizalofop-p-Ethyl (QPE) added to concentrations 1. 5 mg/L, 3 mg/L and 5 mg/L for 72 h. Tap water was used as negative control group, cyclophosphomide (5 mg/ml) was used as positive control, application groups were determined with 3 different concentrations of Quizalofop-p-Ethyl herbicide. Bean seeds were treated with three different doses of Quizalofop-p-Ethyl (1.5mg/L, 3mg/L and 5mg/L) for 72 hours. The studies were carried out in 3 repetitions to the groups at 22˚C.

2.2. Micronucleus (MN) test

After 72 h of incubation, the roots were fixed with acetic acid–ethanol [1:3 (v/v)] solutions for 24 h. The solutions were freshly prepared before use. Both positive and negative control samples were fixed at +4^{0}C the same time. For slide preparation and microscopic examination, the rinsed root tips were hydrolyzed in 1 M HCl at 60^{0}C for 8 min. After staining with Feulgen, they were washed in ultradistilled water. An aliquot of 1 mm of the mitotic zone from well-stained root tips were immersed in glycerin–gelatin (1/7/7(v/v/v) gelatin/ultra distilled water/Glycerin) on a clean slide and squashed under a cover glass. For the analysis of micronuclei, in V.faba, 6000 cells per seedling were scored to calculate MN frequency. The cells with MN were evaluated under 1000 X magnification with use of a light microscope (Japan, OLYMPUS). The following criteria for MN analyses were used in V.faba root tip cells (Tolbert et al., 1992). MN should: (i) be almostly one-third the diameter of the main nucleus; (ii) be on the same plane focus; (iii) have a chromatin structure similar to that of the main nuclei; (iv) be smooth, oval or round shape; and (v) be clearly separated from the main nucleus.

2.3. Mitotic Index

MI was determined by counting the number of mitotic cells among the total amount of scored cells per seedlings. Approximately the frequencies of prophase, anaphase, metaphase and telophase were scored from 3000 cells of three separate seedlings for each treatment and control group. Each experiment was run with three replications.

2.4. Statistical analysis

Data were evaluated by ANOVA using SPSS for Windows software package. Multiple comparisons were performed by Least Significant Difference (LSD) test. $P<0.05$ was considered as level of significance.

3. RESULTS

The MN frequencies and MI values are depicted in Table I. QPE induced the MN formation in V.faba (Figure 1). There is a statistically significant difference between treatment and control groups ($P<0.05$ and $p<0.01$ for 3 mg/mL and 5 mg/ml respectively) for MN frequency except for the concentration of 1,5 mg/mL and statistical analysis refer a statistical difference between 1 and 5 mg/mL and, ($P < 0.01$). It is observed that the increase in MN frequency is dose-dependent. QPE solutions decreased MI in V.faba. QPE at the tested concentrations induced a dose-dependent decrease in the MI and statistical analysis showed that there a statistically significant difference between treatment, negative and positive control groups ($P < 0.001$). MI and prophase stages decreased remarkably at the high concentrations (3 mg/mL and 5mg/ml). Particularly, metaphase, anaphase and telophase stages were not observed in V.faba treated with 5 mg/ml concentration of QPE solutions compared with negative control.

Table 1. Frequency of MN, MI values in V.faba treated with Quizalofob-P-Ethyl (QPE)

Treatments	Concentrations	MN	MI
Negative Control	Tap Water	0,00±0,00	107,3±1,45
QPE	1.5 mg/L	0,66±0,33	99,6±2,72*
	3 mg/L	1,66±0,33	86,6±2,02**
	5 mg/L	2,33±0,33*	70,6±1,76***
Positive Control	Cyclophosphamide 5µg/mL	9,00±0,57***	54,3±1,20***

QPE: Quizalofob-P-Ethyl; MN: Micronucleus; MI:Mitotic Index; *p<0.05, **p<0.01, ***p<0.001

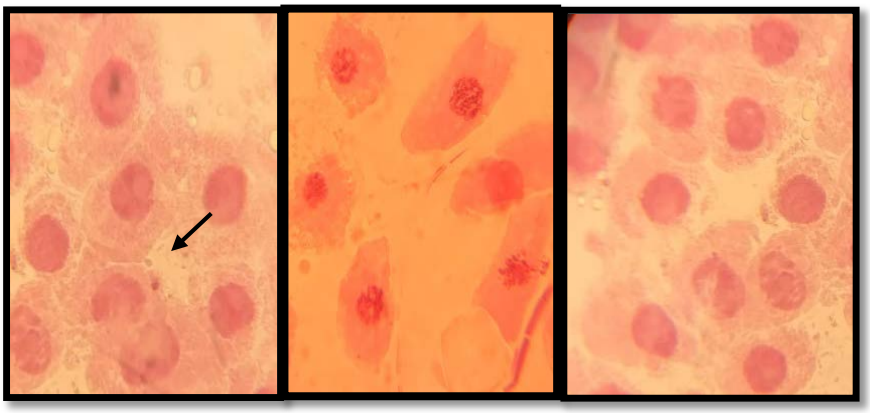

Figure 1: Arrow indicates micronucleated cells in V.faba root tips

4. DISCUSSION

Herbicides, one of the pesticides, are used in agriculture to control weeds. QPE is a phenoxy herbicide compound. It is rapidly absorbed from leaf surfaces and rapidly hydrolyzed to fluazifop acid. The acid is mainly transported in the phloem and accumulates in meristems, where it impairs lipid synthesis in susceptible species (Urano 1982, Erlingson 1988). The indiscriminate use of herbicides in agriculture and the increase in pollution in ecosystems due to industrial development justify the assessment of the toxicity of these chemicals (Marcano et al. 2004). In this study, the pesticide containing Quizalofop-p-ethyl caused an increase in MN frequency in Vicia faba. The statistical difference in the MN value in the ANOVA test reached the level of significance in the LSD results. Quizalofop-p-ethyl herbicide has an inhibitory effect on mitotic division. The statistical difference of the effect on mitotic division seen in the ANOVA test reached the level of significance since $P<0.05$ in LSD results.

In recent studies, the micronucleus test has been used, which indicates that pesticides, including herbicides, reflect chromosomal damage in plant stem cells. Unyayar et al. (2006) Sunyayar et al. investigated the oxidative damage and genotoxicity of cadmium on allium cepa and vicia faba stem cells and they reported an increase in MN frequency and MDA level in parallel with the increase in cadmium dose. They determined a decrease in MI value in both plant stem cells. Mahakavi et al. (2014) investigated to assess the effect of herbicide (quizalafop-p-ethyl) on growth, photosynthetic pigments, enzymes and yield of Black gram (Vigna mungo L.) during the summer season 2013-2014. They reported that 1%, 1.5%, and 2% herbicide application decreased growth, photosynthetic pigment and enzyme production, and the best applied herbicide concentration was 0.5%. In the study of Aksoy et al., the phytotoxic effects of a phenoxy herbicide, Quizalofop-P-Ethyl (QPE, ethyl (R)-2-[4-[(6-chloro-2-quinoxalinyl)oxy]phenoxy]propionate) were investigated on Glycine max L. The effective concentration (EC50) value was determined to be approximately 0.4 M. Morphological and anatomical experiments were performed on 5^{th} and 10^{th} days using QPE concentrations of 0.4 M (EC50) and 0.8 M (EC50x2), with a control for each combination. QPE concentrations were applied by spraying method at 2-3 leaf period. Phytotoxic effects were determined by morphological and anatomical experiments. Root and seedling development, chlorophyll and carotenoid content, and root and seedling anatomy were defined. It has been reported that QPE treatment significantly reduces the amount of carotenoid and chlorophyll b pigment, excluding chlorophyll a. In parallel with the increase in QPE concentration, a decrease in root and seedling length and also changes in the height of the anatomical parts of the seedling compared to the control group were reported.

In a study performed by Karaismailoğlu et al., the genotoxic effects of the herbicide Quizalofop-p-ethyl (QPE) were investigated on the root meristem cells of sunflowers (Helianthus annuus). In the sunflower root growth tests, the effective concentration (EC50) value was determined as approximately 1.5 mg/L. The roots were treated with 0.75 (EC50/2), 1.5 (EC50), and 3 mg/L (EC50 x2) concentrations of Quizalofop-p-ethyl for 24, 36, and 48 h, with a control for each combination. It is reported that Quizalofop-p-ethyl herbicide lead to morphological changes and the formation of micronucleus and decrease in mitotic index/increase in mitotic abnormalities as disturbed prophase, c-mitosis, stickiness, laggard chromosomes, and chromatid bridges with the increase in the herbicide concentration at each exposure time. Cytotoxic effects of environmental pollutants, such as pesticides may be evaluated by analyzing macroscopic (root growth decrease) as well as cytological parameters (types and frequencies of chromosome aberrations and mitotic index as cell kinetic) (Smaka-Kincl et al. 1996). Our study showed that mitotic index in root cells of Vicia faba decreased with increasing QPE concentrations This finding indicates that QPE caused an inhibition of growth in a concentration-dependent manner. When analyzing our results, we found a high incidence of the effects of QPE on the meristematic cells that had compromised the morphology. Inhibition of mitotic activities is often used for tracing cytotoxic substances (Linnainmaa et al. 1978). The mitotic index was significantly reduced with the increase in QPE concentrations, as compared to the control for each application time.

5. CONCLUSION

It is very important to verify that the use of QPE should be subject to control, as it can have a toxic effect on the farmer and the people who consume the plant. In order not to disturb the ecological balance and not to affect the biodiversity, more importance should be given to alternative applications instead of using herbicides. It also points out the importance of phytotoxicity testing of applied chemicals such as pesticides before use.

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