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Genotoxicity of Fenpropathrin and Fenitrothion on Root Tip Cells of *Vicia faba*

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Abstract The genotoxicity of fenpropathrin and fenitrothion on root tip cells of *Vicia faba* was studied. The symptoms were investigated about the mitotic index, the micronucleus frequency and chromosomal aberration frequency of root tip cells of *Vicia faba* which were induced by different concentrations of fenpropathrin and fenitrothion (1×10^{-10}–1×10^{-2} g L^{-1}). Results showed that fenpropathrin and fenitrothion could induce the micronucleus of root tip cells of *Vicia faba*. It occurred in a dose-dependent manner. Peaks were observed at 1×10^{-6} g L^{-1} fenpropathrin and 1×10^{-4} g L^{-1} fenitrothion, and micronucleus frequency reached 14.587 ± 1.511‰ and 14.164 ± 1.623‰, respectively. From 1×10^{-10} g L^{-1} to 1×10^{-6} g L^{-1} fenpropathrin and 1×10^{-4} g L^{-1} fenitrothion, the micronucleus frequency increased with the increase of the concentrations, but beyond this range, the micronucleus frequency decreased with the further increase of the concentrations. A similar trend was observed for mitotic index. Moreover, fenpropathrin and fenitrothion could induce various types of chromosome aberration, such as lagging chromosomes, chromosome fragment, chromosome bridge, multipolar, nuclear buds, karyorrhexis, etc.

Keywords *Vicia faba* · Fenpropathrin · Fenitrothion · Micronucleus frequency · Mitotic index · Chromosome aberration frequency

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Pesticides constitute a heterogeneous category of chemicals which are designed of the control of pests, weeds or plant diseases and have become indispensable in modern agriculture (Ergene et al. 2007; Grisolia 2002). Pesticide residues and accumulation in soil, atmosphere and ground water have seriously affected agricultural productivity, destroyed the ecological environment, damaged biodiversity and restricted agricultural development. Animal, plant and human beings will have been hurt directly or indirectly. In most cases, chronic health problems have long latency periods in human beings and may take many years to manifest themselves. Thus, it is important to study cytogenetic toxicity of pesticides. However, most of studies focused on pesticide runoff, accumulation and movement of pesticide residues in soil and the contamination of ground water by pesticides (Ryals et al. 1998).

Root tip cells of *Vicia faba* are characterized by active metabolism, short division cycle, continuous DNA duplication, enzymatic synthesis, etc. At cells division phase, cells show sensitiveness to some environment pollutants. When there are some pollutants in circumstance, chromosome may happen to be disrupted and mitosis is not finished. Chromosome fragments or whole chromosomes that are left behind during mitotic division appeared in the cytoplasm of the divided cells and formed micronucleus (MN) (Ergene et al. 2007). Micronucleus assay is a rapid method for monitoring the cellular genetic effect of contamination (Grisolia 2002; Matter and Schmid 1970; Terradas et al. 2010) and has been applied routinely to detect chromosome damage and DNA duplication confusion induced by pollutant (Ma et al. 2010; Meng et al. 2002; Zhu et al. 2004). Both fenpropathrin and fenitrothion belong to contact insecticides used widely in agriculture. However, a little information is available about cytogenetic toxicity of fenpropathrin and fenitrothion. Therefore, in the

current research, we analyzed the effects to plant cytogenetic toxicity of different fenpropathrin and fenitrothion concentrations on the micronucleus frequency, the mitotic index, and chromosomal aberration of *Vicia faba* root tip cells with micronucleus assay.

Materials and Methods

In the present work, the higher fenpropathrin or fenitrothion concentration was obtained respectively, according to original concentration of solution 1×10^{-2} g L^{-1} supplied by Shenyang Agriculture Determination Center. Taking into consideration the amount of the active compound contained in the commercial product, the other concentrations were diluted gradually from the higher concentration.

The *Vicia faba* seeds were surface sterilized with 0.5% (v/v) sodium hypochlorite and soaked in distilled water solutions for 24 h. After sufficient imbibitions, seeds were transferred in trays and cultured at 23°C. Distilled water was changed once every 12 h. When their length was about 1 cm, some roots were cultured in different fenpropathrin and fenitrothion solutions (1×10^{-10}, 1×10^{-8}, 1×10^{-6}, 1×10^{-4}, 1×10^{-2} g L^{-1}) for 6 h, respectively; Some roots were cultured in distilled water for 6 h as negative control; The other roots were cultured in 4 μg mL^{-1} cyclophosphamide (CP) solution for 6 h as positive control. After that, the roots were washed in distilled water and recultured for 24 h.

The root tips were excised about 1 cm from recultured seeds during cell division crest-time. Then the root tips were soaked in Carnoy's fixation fluid (anhydrous ethanol: glacial acetic acid = 3:1, V/V) for 24 h, and kept in 70% ethanol in refrigerator at 4°C.

The root tips were first soaked in distilled water for 5 min; then decollemented in 1 M HCl for 13 min at 60°C; after HCl treatment, soaked them in distilled water for 1 min; finally stained with Feulgen.

The micronucleus frequency, cell mitotic index and chromosomal aberration frequency were examined and counted microscopically on squashes. Ten root tips (2,000 cells per root tip) were used in each treatment. The micronuclei were counted only when their diameter did not exceed 1/3 of the main nucleus and when they were localized inside the cell wall and in the cytoplasmatic area surrounding the main nucleus (Marco et al. 1992). SPSS was used for analysis of variance (SPSS 11.0 version).

Results and Discussion

Micronucleus (MN) analysis on *Vicia faba* root tip cells is one of the most useful methods to assess genetic effects of chemicals. In the current study, micronucleus frequency of *Vicia faba* root tip cells induced by different fenpropathrin concentrations was apparently higher than that of the negative control (Table 1). Micronucleus frequency relatively increased when fenpropathrin concentration varied from 1×10^{-10} g L^{-1} to 1×10^{-6} g L^{-1}. A peak was observed at 1×10^{-6} g L^{-1} fenpropathrin and micronucleus frequency reached 14.587 ± 1.511‰ ($p < 0.01$), but micronucleus frequency decreased gradually afterward. A similar trend was also observed in the treatment of different fenitrothion concentrations (Table 1). In the case of fenitrothion treatment, the peak in micronucleus frequency was observed at 1×10^{-4} g L^{-1} fenitrothion instead of 1×10^{-6} g L^{-1} fenitrothion. A maximum of micronucleus frequency of 14.164 ± 1.623‰ was obtained. The formations of micronuclei are likely the consequence of vagrant chromosomes and fragments (Briand and Kapoor 1989). Especially, within a certain range of concentrations, the micronucleus frequency increased with the increase of the concentrations of fenpropathrin and fenitrothion. This seemed that DNA damage was strengthened with the increase of the concentrations of fenpropathrin and fenitrothion. And consequently, the highly concentrated fenpropathrin and fenitrothion had higher micronucleus frequency than that of the low concentrated fenpropathrin and fenitrothion. But beyond this range, micronucleus frequency decreased with the further increase of the concentrations of fenpropathrin and fenitrothion. It implied that the highly concentrated fenpropathrin and fenitrothion solutions further strengthened the cell toxicity and seriously disturbed normal metabolic activity of cells. Moreover, it effectively prevented polymerization and depolymerization of cell intracellular canaliculi and destroyed the formation of spindle apparatus and cell plate. Mitosis was not finished and cells were kept staying division phase, which resulted in decreasing of micronucleus frequency.

The mitotic indexes of *Vicia faba* root tip cells treated with different fenpropathrin concentrations were higher than that of the negative and positive control (Table 1). The mitotic indexes of *Vicia faba* root tip cells increased when fenpropathrin concentration varied from 1×10^{-10} g L^{-1} to 1×10^{-6} g L^{-1}. The mitotic index of 1×10^{-6} g L^{-1} fenpropathrin treatment was the highest, which was 12.57 ± 2.03%. When treatment was above 1×10^{-6} g L^{-1} fenpropathrin, the mitotic indexes began to decrease. Compared with fenpropathrin, the mitotic index of 1×10^{-4} g L^{-1} fenitrothion treatment was the highest, which was 12.20 ± 1.15% (Table 1). The mitotic indexes of 1×10^{-6} g L^{-1} and 1×10^{-4} g L^{-1} fenitrothion treatment were significant higher than that of negative and positive control ($p < 0.05$, $p < 0.01$). There were some factors affecting root tip cells division, for

Table 1 Effects of fenpropathrin and fenitrothion on micronucleus frequency, mitotic index and chromosome aberration of root tip cells of *Vicia faba*

Pesticides	Concentration g L^{-1}	Micronucleus frequency $\bar{X} \pm S$ (‰)	Mitotic index $\bar{X} \pm S$ (%)	Chromosome aberration frequency $\bar{X} \pm S$ (%)
Controls	Negative control	6.082 ± 1.164	8.56 ± 1.06	0.281 ± 0.067
	Positive control	11.64 ± 1.643	8.15 ± 2.08	0.406 ± 0.118
Fenpropathrin	1×10^{-10}	7.785 ± 0.271	8.67 ± 1.77	0.432 ± 0.136
	1×10^{-8}	8.167 ± 1.033	9.27 ± 0.42	0.589 ± 0.16*
	1×10^{-6}	14.587 ± 1.511**	11.57 ± 2.03**##	0.769 ± 0.201**##
	1×10^{-4}	12.688 ± 1.224**	9.06 ± 1.62	0.848 ± 0.173**##
	1×10^{-2}	9.59 ± 1.14*	8.24 ± 1.16	0.486 ± 0.14
Fenitrothion	1×10^{-10}	8.176 ± 1.381	8.84 ± 1.70	0.408 ± 0.081
	1×10^{-8}	11.365 ± 2.28*	9.36 ± 0.68	0.494 ± 0.14
	1×10^{-6}	13.559 ± 2.01**	11.08 ± 1.24*#	0.641 ± 0.147**#
	1×10^{-4}	14.164 ± 1.623**	12.20 ± 1.15**##	0.693 ± 0.125**#
	1×10^{-2}	10.378 ± 1.7*	7.56 ± 1.11	0.394 ± 0.112

*, ** Compared with negative control, $p < 0.05$, $p < 0.01$; #, ## Compared with positive control, $p < 0.05$, $p < 0.01$

example, interphase prolonged in cell division leaded to prolongation of the whole cell cycle. This made mitotic indexes decrease. Shahin et al. (1991) reported that if trigger protein, which could make cells enter S phase, was systhesized enough in G1 phase, cells entered successfully next cell cycle. Within a certain range of concentrations, the mitotic indexes of *Vicia faba* root tip cells increased with the increase of the concentrations of fenpropathrin and fenitrothion. When it reached a peak, mitotic indexes began to decrease gradually. It seemed that the lowly concentrated fenpropathrin and fenitrothion prolonged cells division time and shortened interval of interphase. So, the whole cell division cycle was shortened and mitotic indexes were higher than that of the negative control. But with the further increase of the concentrations of fenpropathrin and fenitrothion, both intervals of interphase and cells division cycle were prolonged, besides mitotic indexes began to decrease. The treatment with highly concentrated fenpropathrin or fenitrothion might disturb or prevent trigger protein synthesis, make cells keep staying at G1 phase and not enter division phase.

The chromosomal aberration frequency of *Vicia faba* root tip cells treated with different fenpropathrin concentrations was higher than that of the negative control (Table 1). The chromosomal aberration frequency gradually increased with fenpropathrin concentration varying from 1×10^{-10} g L^{-1} to 1×10^{-4} g L^{-1}. The 1×10^{-4} g L^{-1} fenpropathrin treatment had the highest chromosomal aberration frequency, which was 0.848 ± 0.173%. The chromosomal aberration frequency of 1×10^{-6} g L^{-1} and 1×10^{-4} g L^{-1} fenitrothion treatment were significantly higher than that of negative and positive control ($p < 0.05$, $p < 0.01$). When fenitrothion treatment was above 1×10^{-4} g L^{-1}, chromosomal aberration frequency decreased. In the present study, chromosomal aberration frequency of different fenitrothion concentrations was similar to fenpropathrin treatment. Namely, a peak was also observed at 1×10^{-4} g L^{-1} fenitrothion. In the present study, many chromosome abnormalities were observed in the whole cell cycle (Figs. 1, 2). These abnormalities were the presence of single micronucleus (Fig. 1a, c, i, l), double micronuclei (Fig. 1b), lagging chromosomes (Fig. 1g, j, m), chromosome fragment (Fig. 1e, k), chromosome bridge (Fig. 1h, m), multipolar (Fig. 1f), chromosome stickiness (Fig. 1d), nuclear buds (Fig. 1n, o), karyorrhexis (Fig. 1p), etc. Spindle dysfunction might mainly be mitotic irregularities which constituted a significant portion of chromosomal aberrations (Tabur and Demir 2010). Laggards and precocious movement of chromosomes might be the result of the failure of spindle apparatus to organize in normal way (Patil and Bhat 1992). Chromosome fragments might lead to clastogenic effect and possible mutagenicity (Fiskesjö 1997). Anaphase bridges could be the result of inversions (Tabur and Demir 2010). Highly concentrated pesticides interfered with chromosomal distribution during cell division so that multipolar occurred. The nuclear buds might be caused by polyploidization events, whose exceeding material was released from the cells (Fernandes et al. 2007). These results showed that fenpropathrin and fenitrothion directly damaged DNA duplication, disturbed RNA transcription and protein synthesis, and some materials which were related to chromosome movement could not be produced. Hence, chromosome aberration happened.

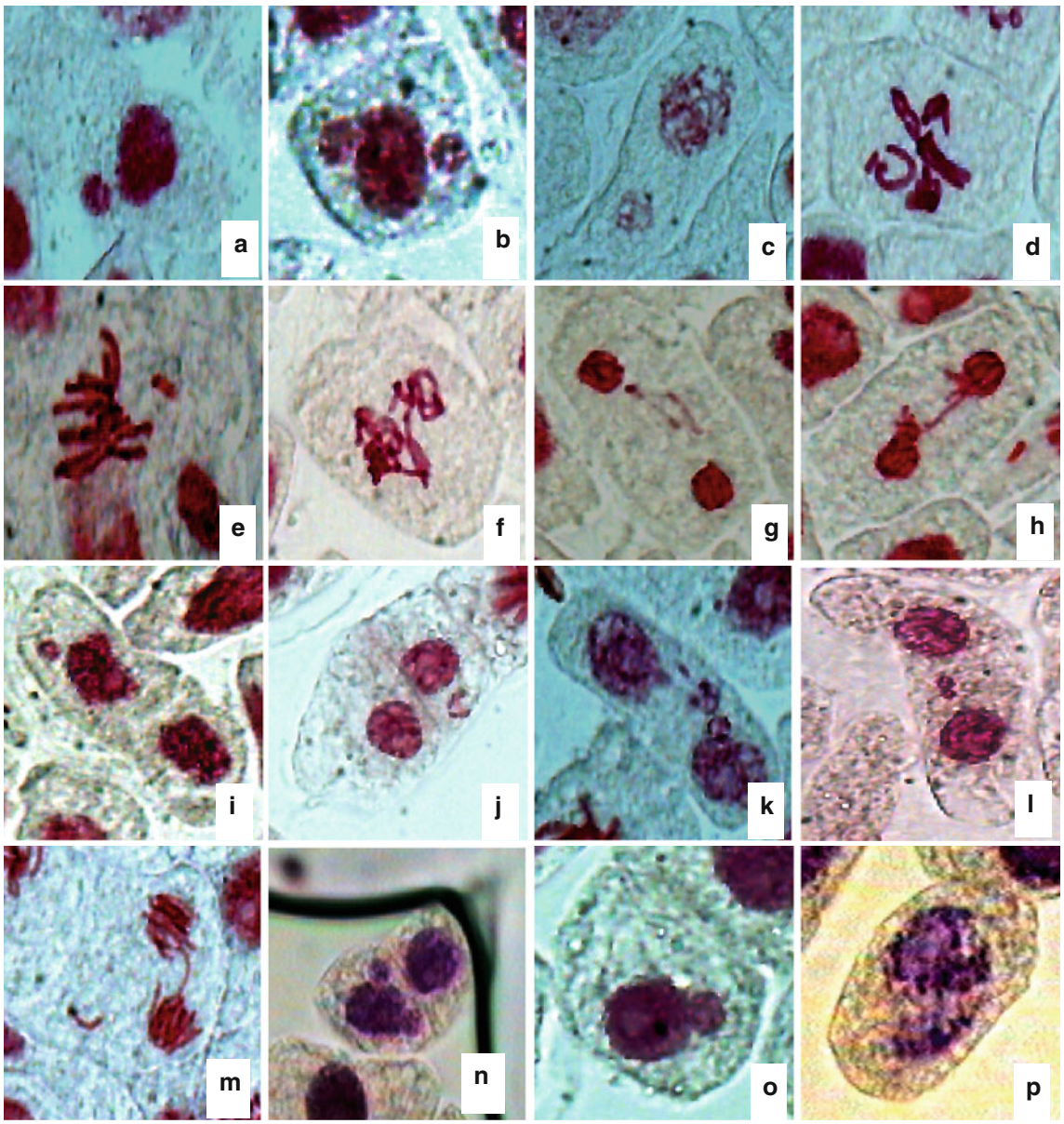

Fig. 1 Chromosomal aberrations of root tip cells of *Vicia faba* germinated in different fenpropathrin and fenitrothion concentrations. The concentrations were 1×10^{-10}, 1×10^{-8}, 1×10^{-6}, 1×10^{-4} and 1×10^{-2} g L^{-1}. **a** Single micronucleus in interphase; **b** Double micronuclei in interphase; **c** Single micronucleus in prophase; **d** Chromosome stickiness and fragment in metaphase; **e** Chromosome fragment in metaphase; **f** Chromosome was divided into two parts in anaphase; **g** Chromosome lagging in anaphase; **h** Chromosome single bridge in anaphase; **i** Single micronucleus in anaphase; **j** Chromosome lagging in telophase; **k** Chromosome fragment and micronucleus in telophase; **l** Single micronucleus in telophase; **m** Chromosome single bridge and lagging in telophase; **n** Nuclear double buds **o** Nuclear single buds; **p** Karyorrhexis

When fenpropathrin or fenitrothion concentration was 1×10^{-2} g L^{-1}, micronucleus frequency, mitotic index and chromosomal aberration frequency was lower than that of 1×10^{-4} g L^{-1}, respectively. It seemed that highly concentrated pollutant had a serious toxic effect on *Vicia faba* root tip cells. Moreover, we also observed that *Vicia faba* root tip apparently darkened under 1×10^{-2} g L^{-1} fenpropathrin and fenitrothion. These results showed that root tip cells were seriously damaged. So, the cells with damaged seriously genetic material could not enter the next cycle and even deceased. This is in agreement with the literature reports (Brunetti et al. 1988).

In conclusion, fenpropathrin and fenitrothion could induce mutagenesis and should be used for optimization of concentration in agricultural manufacturing. The research objective was to have a higher effect of killing insect and lower mutagenesis.

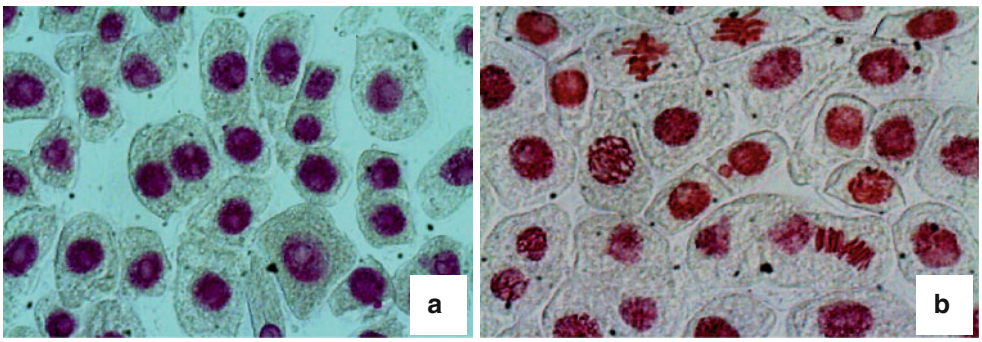

Fig. 2 Chromosomal aberrations of root tip cells of *Vicia faba* germinated in distilled water (**a**) and 4 μg mL^{-1} cyclophosphamide (CP) (**b**). Distilled water was as negative (control); cyclophosphamide (CP) was as positive control

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References

Briand CH, Kapoor BM (1989) The cytogenetic effects of sodium salicylate on the root meristem cells of *Allium sativum* L. Cytologia 54:203–209

Brunetti R, Majone F, Gola I, Beltrame C (1988) The micronucleus test: examples of application to marine ecology. Mar Ecol Prog Ser 44:65–68

Ergene S, Çelik A, Çavaş T, Kaya F (2007) Genotoxic biomonitoring study of population residing in pesticide contaminated regions in Göksu delta: micronucleus, chromosomal aberrations and sister chromatid exchanges. Environ Intern 33:877–885

Fernandes TCC, Mazzeo DEC, Marin-Morales MA (2007) Mechanism of micronuclei formation in polyploidizated cells of *Allium cepa* exposed to trifluralin herbicide. Pestic Biochem Phys 88:252–259

Fiskesjö G (1997) Allium test for screening chemicals; evaluation of cytological parameters. In: Wang W, Gorsuch JW, Hughes JS (eds) Plants for environmental studies. Lewis Publishers, New York, pp 308–333

Grisolia CK (2002) A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. Mutat Res 518:145–150

Ma LJ, Zhang Y, Bu N, Wang SH (2010) Alleviation effect of alginate—derived oligosaccharides on *Vicia faba* root tip cells damaged by cadmium. Bull Environ Contam Toxicol 84:161–164

Marco AD, Simone CD, Raglione M, Testa A, Trinca S (1992) Importance of the type of soil for the induction of micronuclei and the growth of primary roots of *Vicia faba* treated with the herbicides atrazine, glyphosate and maleic hydrazide. Mutat Res 279:9–13

Matter BE, Schmid W (1970) Tremimon-induced chromosomal damage in bone marrow cell of six mammalian species evaluated by the micronucleus test. Mutat Res 12:417–418

Meng Z, Sang N, Zhang B (2002) Effects of derivatives of sulfur dioxide on micronuclei formation in mouse bone marrow cells in vivo. Bull Environ Contam Toxicol 69:257–264

Patil BC, Bhat GI (1992) A comparative study of MH and EMS in the induction of chromosomal aberrations on lateral root meristem in *Clitoria ternetea* L. Cytologia 57:259–264

Ryals SC, Genter MB, Leidy RB (1998) Assessment of surface water quality on three eastern North Carolina golf courses. Environ Toxicol Chem 17:1934–1942

Shahin SA, El-Amoodi KH (1991) Induction of numerical chromosome aberrations during DNA synthesis using the fungicides nimrod and rubigan-4 in root tip of *Vicia faba* L. Mutat Res 261:169–176

Tabur S, Demir K (2010) Role of some growth regulators on cytogenetic activity of barley under salt stress. Plant Growth Regul 60:99–104

Terradas M, Martín M, Tusell L, Genesca A (2010) Genetic activities in micronuclei: Is the DNA entrapped in micronuclei lost for the cell? Mutat Res 142:60–67

Zhu Y, Wang J, Bai Y, Zhang R (2004) Cadmium, chromium, and copper induce polychromatocyte micronuclei in carp (*Cyprinus carpio* L.). Bull Environ Contam Toxicol 72:78–86