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Mutagenic and Cytotoxic Effects of Pesticide Lambada 5EK (Lambda-Cyhalothrin) on Sweet and Hot Pepper (Capsicum annuum L.), Beetroot (Beta vulgaris) and Onion (Allium cepa) In Vivo

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Abstract. The aim of the present work was to study the potential mutagenic and cytotoxic effects of synthetic pyrethroid insecticide Lambada 5EK in the root meristems of some vegetables. Two concentrations of the pesticide were investigated (0.03% - recommended in agricultural practices, and tenfold lower concentration - 0.003%), and three plant-species, widely used as food, were tested - sweet and hot pepper (*Capsicum annuum* L.), beetroot (*Beta vulgaris* L.) and onion (*Allium cepa* L.). The results obtained regarding the influence of pesticide on mitotic cell division and chromosome status showed that the species have different sensitivity towards the pesticide action. The hot pepper was the most sensitive of all the tested plant species towards the impact of the pesticide in mitosis, and lower mitotic index values were recorded in both tested concentrations compared to the control. The most common observed chromosomal aberrations were: anaphase/telophase bridges, lagging acentric chromosome and/or chromosome fragments, and cells with micronuclei; they were found in all tested plant species, except beetroot treated with 0.003% solution of the pesticide. These results suggested that the beetroot possesses some endurance to the mutagenic action of the insecticide and probably it is an appropriate culture in crop rotation in agricultural practice when lambda-cyhalothrin pesticides have been used.

Key words: genotoxicity, mitotic index, chromosomal aberrations, micronuclei, lambda-cyhalothrin, vegetables.

Introduction

In a number of cases, classified as harmless pesticides and their degradation products possess increasing toxicity, as they move up through the trophic pyramid; in the high trophic level, their concentration could increase to 1000 times (Owen & Chiras, 1990).

Some of these chemicals are promutagens and they are activated metabolically after getting into the body, and some are only activated in plants. When they get into a human organism with plant food, they may become a real genetic danger to humans. A number of authors have identified cytogenetic, clastogenic and cytotoxic effects of some agro pesticides (Özkan *et al*., 2009; Asita & Hatane, 2012; Asita & Mokhobo, 2013; Mesi & Kopliku, 2013; Özkara *et al*., 2015).

It is really essential to find the proper plant test-systems to evaluate the risk of genotoxicity for examining such mutagenicity compounds. Apart from the recommended pesticide

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concentrations, it is also important to examine significantly lower concentrations, because the residual amounts of pesticides do not disappear in the soil; they fall into the groundwater and enter into plants through roots.

Lambada 5EK is a synthetic pyrethroid insecticide with lambda-cyhalothrin (50 g/l) active compound. In agricultural practices, this pesticide is used against vermin as *Eurygaster integriceps* (cereals), *Cydia pomonella* (apples), *Lobesia botrana* (vineyards), *Anarsia lineatella* (apricots), *Myzus persicae* (pepper), *Aphys* spp. (cucumbers), *Leptinotarsa decemlineata* (potatoes), etc.

The purpose of the present study is to estimate the mutagenic and cytotoxic effects of lambda-cyhalothrin on root-tip meristematic cells of four plant test-systems that are widely used as food.

Materials and Methods

It was studied two concentrations of Lambada 5EK: 0.03% solution (used in agricultural practices) and 0.003% solution.

In the present study were tested 4 plant's test-systems: sweet and hot pepper (*Capsicum annuum* L.; cultivars Kurtovska kapia and Biala shipka respectively), beetroot (*Beta vulgaris*) and onion (*Allium cepa*; cultivar Density). The seeds were cultivated in Petri dishes with distilled water (control) or pesticide solutions till seed germination. The primary root apex was excised (3-5 mm), washed (in distilled water) and fixed (3:1 ethanol: glacial acetic acid). The fixed roots were washed in ethanol (96% and 70%), hydrolyzed in 3 N HCl (10 min) and treated in 45% CH_3COOH (30 min) (by STAYKOVA *et al.*, 2005). The roots of beetroot were hydrolyzed for 40 min (in 3 N HCl) and treated for 90 min in 45% CH_3COOH (authors' modification). The pepper and onion roots were stained with 4% acetic-carmine for 2 h with heating. The roots of beetroot were stained without heating for 3 days (authors' modification). The effects of pesticide were studied on squash preparations and there were analyzed 3000 cells of each variant (magnification 400 ×).

The intensity of cell division was determined by calculation of mitotic index (IM) (number of dividing cells per total analyzed cells, in percent). The frequencies of mitotic phases were calculated also (phase index - number of cells in concrete mitotic phase per total dividing cells, in percent).

To evaluate the cytotoxicity of the tested concentrations of pesticide, the IM of the treated cells were compared with those of the control.

It was applied an F-test Two-Sample for Variances (ANOVA) to test if the variances between the controls and the treated samples were statistically significant and reliable. If there is a significant difference in a variant, it was applied the t-Test: Two-Sample Assuming Unequal Variances. In other cases, it was used t-Test: Two-Sample Assuming Equal Variances.

The mutagenic effects (genotoxicity) of the tested pesticide concentrations were examined by investigation of chromosome aberrations (chromosome fragments, acentric chromosomes, anaphase- and telophase-bridges) and micronuclei in the treated cells and in the controls.

Results and Discussion

The results of the investigation of the cell division are presented in Table 1. The data showed that IM was higher in all controls than in the treated variants with a 0.03% solution. This indicates that Lambada 5EK slows down the cell division in this concentration in the studied plant species. The difference between IM in control and treated material was the greatest in hot pepper (63.47% and 54.70%, respectively), and the smallest in beetroot (51.60% and 49.77%, respectively). In the tested plant species (except the hot pepper), the concentration of 0.003% of pesticide slightly stimulated cell division and IM was higher than in control. In the sweet pepper, the prophase index (IPph) was higher in treated material than in control and the indexes of metaphase (IMph), anaphase (IAph) and telophase (ITph) were lower. These results indicated retention of dividing cells in prophase after treatment and delayed the transition to the next stages of mitosis. In the hot pepper, there was established a substantial reduction of mitotic index in the treated material with both doses of pesticide. Higher indexes of telophase were also found in the treated material (1.37% and 1.77% after treatment with 0.003% and 0.03% Lambada, respectively, while 0.16% in control). These results indicate accumulation of cells in prophase probably due to the slower cytokinesis after pesticide treatments. The delay in mitosis was found in beetroot and onion as well (Table 1). Similar mitodepressive actions of lambda-cyhalothrin on the mitotic index in *Allium cepa* as well as in mice have been reported by other authors (MALIK, 2013; ÇAVUŞOĞLU *et al*., 2014).

Decreasing in the intensity of cell division, after treatment with different pesticides, has been reported in other plants tested (RUFUS *et al*., 2000; AYDEMIR *et al*., 2008).

The pesticide concentration is cytotoxic when IM of treated cells is ≤ ½ of the control IM (ASITA & MATEBESI, 2010). Our findings indicated that 0.03% Lambada is not cytotoxic in the investigated plant species. The effects of this pesticide on mitosis in sweet and hot peppers, beetroot and onion showed that the species have a different value of sensitivity. The results showed that hot pepper is the most sensitive to the action of the pesticide among tested species.

In the treated cells, the following types of chromosomal aberrations were registered: anaphase (telophase) bridges (intact or broken) (Fig. 1), lagging acentric chromosome and/or chromosome fragments (Fig. 2), and cells with micronuclei (Fig. 3).

The data of chromosomal aberrations, observed in the cells treated with Lambada 5EK, are presented in Table 2. In the controls, there were not any chromosomal disorders identified, which indicates that there is no auto mutagenesis.

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Table 1. Mitotic and phase indices (in %) in sweet and hot peppers, beetroot and onion, treated with different concentrations of the pesticide Lambada 5EK. (n = 6; * indicate significant difference at p≤0.05 between the treated and control variants; n.s. indicate non-significant difference at p>0.05).

Plant Species	Samples	Mitotic Index, IM	Phase Indices			
			IPph	IMph	IAph	ITph
Sweet pepper	Control	64.10	86.84	5.82	4.52	2.86
	0.003%	66.03*	93.54*	3.43*	2.27*	0.76*
	0.03%	59.47*	91.98*	4.32*	3.25*	0.50*
Hot pepper	Control	63.47	86.82	6.72	6.25	0.16
	0.003%	56.07*	88.35$^{n.s.}$	5.35*	4.93*	1.37*
	0.03%	54.70*	86.35$^{n.s.}$	5.12*	6.83*	1.77*
Beetroot	Control	51.60	92.25	3.17	2.58	1.49
	0.003%	57.17*	92.48$^{n.s.}$	4.14$^{n.s.}$	2.80$^{n.s.}$	0.64*
	0.03%	49.77*	95.31*	2.61$^{n.s.}$	1.34*	0.74*
Onion	Control	60.43	94.93	2.48	1.27	1.32
	0.003%	63.83$^{n.s.}$	92.85*	2.77$^{n.s.}$	2.77*	1.67$^{n.s.}$
	0.03%	56.57*	94.87$^{n.s.}$	2.47$^{n.s.}$	1.41$^{n.s.}$	1.24$^{n.s.}$

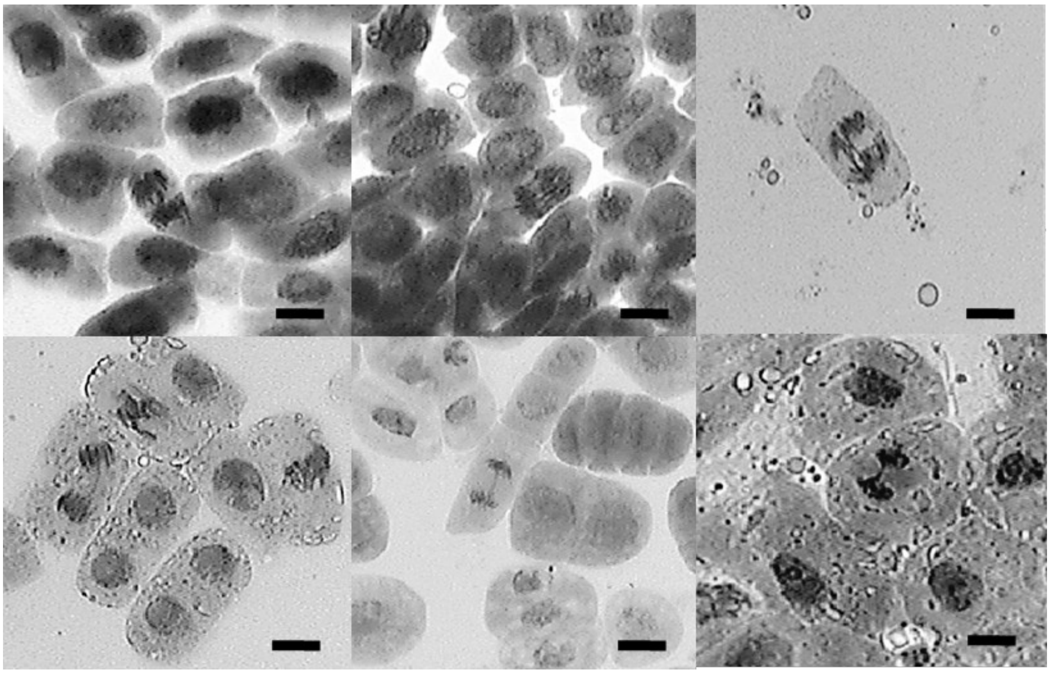

Fig. 1. Anaphase (telophase) bridges induced in tip root cells in sweet and hot peppers, beetroot and onion treated with Lambada 5EK (magnification 400 ×), scale bar 10 μm.

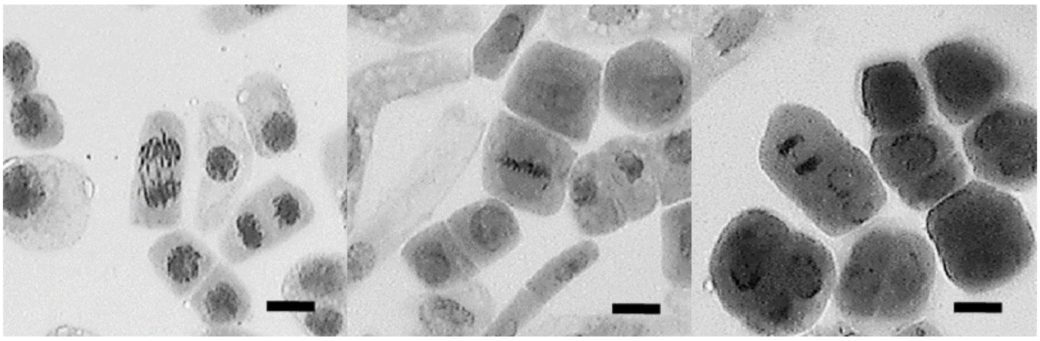

Fig. 2. Chromosomal fragments and acentric chromosomes induced in tip root cells in onion treated with Lambada 5EK (magnification 400 ×), scale bar 10 μm.

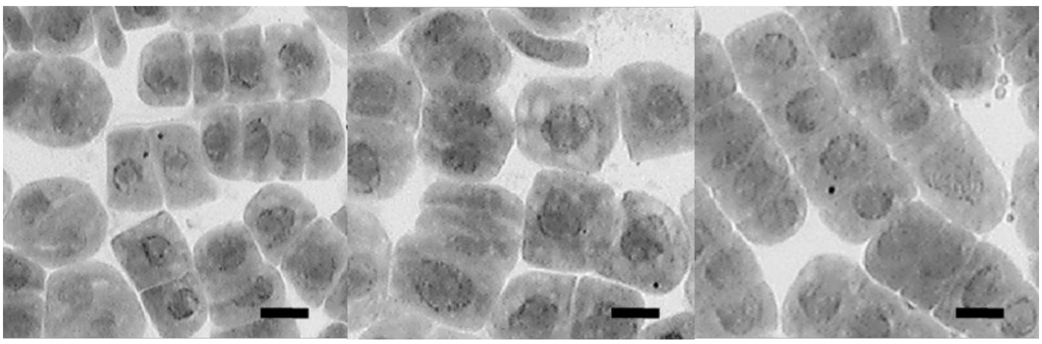

Fig. 3. Micronuclei induced in tip root cells in sweet and hot peppers, beetroot and onion treated with Lambada 5EK (magnification 400 ×), scale bar 10 μm.

Chromosomal aberrations were observed in the treated variants, except beetroot treated with 0.003% solution of pesticide. Higher values of the total frequency of chromosomal aberrations, as well as the frequency of aberrations in dividing cells, were recorded after treatment with the recommended concentration in agriculture (0.03%). The highest percentages of cells with induced mutagenesis were found in onions (0.50% of cells analyzed, and 0.88% of dividing cells, Table 2).

After treatment with the higher concentration of the pesticide, there was registered mostly micronuclei in plant cells. Lagging acentric chromosomal fragments and/or chromosomes were observed only in cells of the onion, treated with 0.03% Lambada, however, we consider that they are also formed in the other tested plants, in which micronuclei were observed.

Table 2. Frequency of chromosomal aberrations in sweet and hot peppers, beetroot and onion treated with Lambada 5EK. (1 – towards the total number of cells (in %); 2 – towards mitotic cells (in %)).

Plant Species	Samples	Chromosomal aberrations		Cells with micronuclei		Chromosomal bridges		Chromosomal fragments and acentric chromosomes	
		1	2	1	2	1	2	1	2
Sweet pepper	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.003%	0.08	0.12	0.00	0.00	0.08	0.12	0.00	0.00
	0.03%	0.38	0.64	0.34	0.57	0.04	0.07	0.00	0.00
Hot pepper	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.003%	0.05	0.09	0.05	0.09	0.00	0.00	0.00	0.00
	0.03%	0.19	0.35	0.16	0.29	0.03	0.06	0.00	0.00
Beetroot	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.003%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.03%	0.17	0.35	0.14	0.28	0.03	0.07	0.00	0.00
Onion	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.003%	0.09	0.13	0.00	0.00	0.09	0.13	0.00	0.00
	0.03%	0.50	0.88	0.23	0.40	0.11	0.20	0.15	0.27

Similar chromosome aberrations in onion after treatment with lambda-cyhalothrin and other pesticides have been observed by other authors (YEKEEN & ADEBOYE, 2013). Anaphase (telophase) chromosome bridges were noted in almost all treated variants. The lowest number of cells with chromosomal aberrations was found in beetroot. After treatment with a diluted solution of the pesticide (0.003%), there were no chromosomal aberrations in cells. These data show that the beetroot possesses somewhat endurance to the mutagenic action of the insecticide.

The highest frequency of cells with micronuclei formed after treatment with a 0.03% Lambada was recorded in sweet pepper (0.34% of cells analyzed and 0.57% of dividing cells), and relatively high – in onion (0.23% of cells analyzed and 0.40% of the dividing cells). The lower pesticide concentration induced micronuclei only in the cells of hot pepper. The highest frequencies of anaphase (telophase) chromosomal bridges were found in cells of onion in both concentrations of pesticide. Chromosome fragments and acentric chromosomes were found only in onions at a higher concentration of the pesticide.

The presented results show that both tested pesticide concentrations have mutagenic effect in the cells of the plants (except beetroot, where lower concentration does not cause mutational changes).

Mutagenic effects of Lambada 5EK occur in the formation of anaphase (telophase) bridges that can be a result from ligated sister chromatids (provoked by a single or double detachment of telomeres) or dicentric and/or polycentric chromosomes. The bridges can break away, with the anaphase progression, and thereby, new acentric fragments, lagging in the equatorial zone of the cell, can be released. Acentric fragments can also be formed as a result of the removal of parts of chromosomes after fragmentations; in daughter cells (next cell generation), acentric fragments or chromosomes form micronuclei observed in the cytoplasm.

It has been reported that Lambda-cyhalothrin possess mutagenic effect in animal species as rats, mice, fishes, rabbits, tadpoles and also in human lymphocyte culture (CAMPANA *et al.*, 1999; FAHMY & ABDALLA, 2001; CAMPANA *et al.*, 2003; CELIK *et al.*, 2003, 2005; NARAVANENI & JAMIL, 2005; VELMURUGAN *et al.*, 2006; BASIR *et al.*, 2011; MURANLI & GÜNER, 2011; MALIK, 2013; MURANLI, 2013; XIA *et al.*, 2013; CAVUSOGLU *et al.*, 2014; GADHAVE *et al.*, 2014; FETOUI *et al.*, 2015).

A similar mutagenic effect of various pesticides has been published by other authors (DIMITROV *et al.*, 2006; AYDEMIR *et al.*, 2008; ASITA & MATEBESI, 2010; MARTÍNEZ-VALENZUELA *et al.*, 2017).

Conclusions

It was found that the concentration of 0.03% (recommended in agriculture) of the pesticide Lambada 5EK slow down the cell division in root meristematic cells of sweet and hot peppers, beetroot and onion. There was found an accumulation of cells in prophase, and a delay in the transition toward the metaphase and next mitotic phases in the treated materials. Mitosis changes showed that the plants possess a different level of sensitivity. The hot pepper showed the highest sensitivity to the pesticide concentrations. There were no cytotoxic effects of the concentrations tested in the plant species, however, there were mutagenic effects. Chromosomal aberrations were found in the plants tested, except in beetroot treated with the lower concentration (0.003%), the lowest genotoxicity was found in beetroot, probably due to some endurance toward the mutagenic action of the insecticide.

In the frequent use of pesticides, the residual amounts of them accumulate in the soil. Basing on the results observed in the present study we assume that beetroot is an appropriate culture in crop rotation in agricultural practice when lambda-cyhalothrin based insecticides have been used in the previous season.

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