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Cytotoxic and mutagenic effects of pesticides Verita WG and Actara 25 WG on sweet pepper (*Capsicum annuum* L.) and onion (*Allium cepa* L.)

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Abstract

Popova, T., Vasileva, P., Stoyanov, I., Ilieva, I., Staykova, T. & Ivanova, E. (2021). Cytotoxic and mutagenic effects of pesticides Verita WG and Actara 25 WG on sweet pepper (*Capsicum annuum* L.) and onion (*Allium cepa* L.). *Bulg. J. Agric. Sci., 27 (3)*, 569–574

The aim of the present study is to investigate the potential mutagenic and cytotoxic effects of insecticide Actara and fungicide Verita in the root meristems of onion (*Allium cepa* L.) and sweet pepper (*Capsicum annuum* L.). Three concentrations of each of these pesticides were investigated (stock solutions used in agricultural practices, and 10- and 20- times lower concentrations). The results showed that the onion and sweet pepper have different sensitivity towards Actara and Verita. The Verita 0.15% solution (recommended for agricultural usage), significantly reduces the pepper seeds germination. Similar effects in pepper seeds were found after treatment with other concentrations of the tested pesticides. In contrast, the treated onion seeds showed overall increased germination. Mitosis-depressive action of both pesticides was found (strongly expressed in onion after Verita 0.15% treatment and pepper after Verita-lowest concentration and Actara 0.02%). Different chromosomal aberrations were observed in the cells: anaphase/telophase bridges, lagging chromosomes and/or chromosome fragments, and micronuclei. Both studied pesticides have a genotoxic effect in the concentrations used in practice as well as in concentration lower than recommended. This indicates that even minimal residual amounts of them, accumulated in the soil, can damage plant chromosomes and could have a real mutagenic effect.

Keywords: pesticides; chromosomal aberrations; micronuclei; cytotoxicity; vegetables; *Capsicum annuum*; *Allium cepa*

Introduction

Nowadays pesticides are widely used in agricultural practice to increase crop yields and control pests and plant diseases. Due to their indiscriminate use, they accumulate in the environment and many non-target species are being exposed to them including humans. Chronic exposure to these pesticides may lead to various pathological conditions and cause damage to human health (van der Werf, 1996; Alavanja et al., 2004; Wang et al., 2016). Some of these chemicals act as pro-mutagens; they activate metabolically after they enter the body but some activate only in plants. When a human consumes such substances with plant foods, those substances may become a real genetic danger. Many authors have identified cytotoxic, genotoxic and mutagenic effects of agro pesticides on different plant and animal species as well as in humans (Ali et al., 2008; Ansari et al., 2009; Omari, 2011; Ozakca & Silah, 2013; Dubey et al., 2015; Fetoui et al., 2015; Martinez-Valenzuela et al., 2017). The pesticide residues that remain in veg-

etables and fruits are potentially harmful to human health. Therefore it is essential to investigate the genetic effects of lower pesticide concentrations in parallel to concentrations used in practice.

The aim of the present study is to investigate the pesticides Verita WG and Actara 25 WG for mutagenic and cytotoxic effects on root-tip meristem cells of sweet pepper (*Capsicum annuum* L.) and onion (*Allium cepa* L.) in concentrations used in agricultural practice as well as 10- and 20- times lower concentrations.

Materials and Methods

In the present study the following concentrations of pesticides were investigated: 0.15% Verita and 0.02% Actara – stock solutions used in agricultural practices, and 10- and 20- times lower concentrations (10x and 20x respectively).

Verita WG contains both Fosetyl Aluminium (667 $g.kg^{-1}$) and Fenamidone (44 $g.kg^{-1}$) as active ingredients. This is a fungicide with systemic and translaminar distribution in plants, with preventive and curative action against mildew on vines, tomatoes, onion, tobacco, potato, and cucumbers.

Actara 25 WG contains Thiamethoxam (250 $g.kg^{-1}$) as an active ingredient. The pesticide belongs to the new chemical class of neonicotinoids, used for the control of Aphids, Whitefly, and Jassids (sucking pests). This is a strong insecticide that is fast-acting and therefore rapidly taken up by the plants. The mode of action is on a specific protein (the nicotinic acetylcholine receptor) in the brain of insects and inhibits the feeding reflex. In the agricultural practice, Actara is applied in many vegetable crops like cucumber, pepper, tomato, potatoes, coffee, rice, cotton, and also ornamentals, and tropical plants.

Two plants test-systems were tested: sweet pepper (*Capsicum annuum* L., cultivar Sivriya), and onion (*Allium cepa* L., cultivar Ispanska Kaba 482). Fifty seeds of pepper and onion were cultivated in Petri dishes in humid conditions with pesticide solution or distilled water (in the control variants) till seed germination. The number of germinated seeds in each experimental variant and in the control over a 20-day period was registered. Total toxicity was calculated as percentage of germinated seeds from the total number of seeds in the control and in the treated samples. The root apexes were excised (3-5 mm), washed (dH_2O), fixed in fixative (ethanol: glacial acetic acid 3:1), washed in ethanol (96% and 70%), hydrolyzed (3 N HCl 10 min), treated in 45% CH_3COOH (30 min) (Staykova et al., 2005), and stained with 4% acetic-carmine with heating (2 h). The effects of the pesticide were studied on squash preparations and there were analyzed at least 2000 cells of each variant (magnification 400x). From each experimental and control samples, were analyzed at least 5 microscope slides prepared from 5 different roots.

The cytotoxic effect of the pesticide solutions was determined by calculating a mitotic index and phase indices. The mitotic index (IM) was determined as a number of dividing cells per total analyzed cells (in percent). The frequencies of mitotic phases were presented as phase indices as a number of cells in the 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 was compared with those of the control.

The mutagenic effects (genotoxicity) of the tested pesticide concentrations were examined by anaphase analysis and micronuclei test. The number of cells with chromosomal fragments and lagging chromosomes, anaphase- and telophase-bridges, and micronuclei in the treated cells and in the controls were registered, and the frequencies in percentages were calculated.

Results and Discussion

The results concerning the overall toxicity of the different concentrations of the pesticides tested are presented in Table 1.

The results show that both plant species react differently to applied pesticides. Onion seeds germination ranges from 64% (the control and the seeds treated with the lowest

Table 1. Influence of different concentrations of Verita and Actara on the germination of *Allium cepa* and *Capsicum annuum* seeds

Sample		Control	Verita			Actara		
			0.15%	10X	20X	0.02%	10X	20X
Allium cepa	Number of seeds	50	50	50	50	50	50	50
	Number germinated seeds	32	36	40	38	42	39	32
	Germination, %	64	72	80	76	84	78	64
Capsicum annuum	Number of seeds	50	50	50	50	50	50	50
	Number germinated seeds	49	27	43	43	45	47	47
	Germination, %	98	54	86	86	90	94	94

Actara concentration) to 84% (the seeds treated with Actara stock solution). Pesticide-treated onion seeds showed overall increased germination compared to the control. In contrast to the onion, in pepper seeds, we recorded the highest germination in the control (98%) and lower values for the treated variants, with the lowest percentage of germinated seeds being observed with a Verita stock solution (54%). In general, the pepper seeds treated with the Verita fungicide had a more pronounced reduced germination than those treated with Actara.

The results show a difference in the sensitivity of onion and pepper seeds to the action of these two pesticides. Apparently, the Verita fungicide solutions and the two higher concentrations of the Actara insecticide have a stimulating effect on onion seeds germination (the percentage of germinated seeds is higher than in the control). But in sweet pepper, the two pesticides depressed germination, the highest total toxicity being found in the Verita stock solution, where the lowest percentage of germinated seeds was recorded (54%). Consequently, the Verita solution 0.15% recommended for practical application significantly reduces the germination of pepper seeds.

In the experiments with seeds of garden peas (*Pisum sativum*) treated with the same concentrations of Verita, no negative effect on the germination has been observed (Stoyanov et al., 2013). Probably these differences in the reactions of the different plant species to the applied Verita and Actara concentrations are due to the specific individual features of the tested plants. The results obtained of the general toxicity studying may be influenced by peculiarities in the germination of the particular plant variety, seed storage in the commercial network, and other factors.

The results concerning the mitotic index and the phase indices in the control and experimental samples treated with the studied pesticides are presented in Table 2.

The mitotic index is an indicator of the rate of cell division. In both tested plant species the highest cell division intensity was recorded in the controls (79.89% onions and 75.40% pepper). Consequently, both studied pesticides slow the rate of cell division in onion and pepper root meristematic cells.

The results indicate that both plant species sweet pepper and onion reacted in a different way to the tested pesticides. In onion, the lowest mitotic indices were found after treatment with Verita stock solution (43.42%) and with the lowest Actara concentration (55.44%). The same pesticide concentrations caused increased prophase indices (Actara 20X – 94.09%; Verita 0.15% – 95.62%) compared with the control (91.53%). The same tendencies were observed in metaphase and telophase indices. A similar delay of mitotic division in prophase was found in the study of the cytotoxic and genotoxic effects of the fungicide Verita on the plant test system *Pisum sativum* (garden peas) (Stoyanov et al., 2013). For sweet pepper, the lowest values in the mitotic index were recorded in the Actara stock solution and the lowest Verita concentration (58.20% and 58.10%, respectively). The highest prophase index was recorded in the control (94.50%) in contrast to the other phases indices (metaphase and anaphase), which were higher in the treated samples. This accumulation of metaphases and anaphases in the treated material may result from difficulties in the mitotic spindle action.

The reduction of the mitotic index as an effect of the treatment of the root meristem with pesticides was also found in the studies of other authors (Gill & Shaukat, 2000; Rufus et al., 2000; Singh et al., 2007; Aydemir et al., 2008;

Table 2. Mitotic index (IM) and phase indices in *Allium cepa* and *Capsicum annuum* control and treated samples, %

Sample		Mitotic Index, IM	Phase Indices			
			IPph	IMph	IAph	ITph
Allium cepa	Control	79.89	91.53	2.89	0.96	4.60
	Actara 0.02%	71.98	91.05	3.11	2.60	3.30
	Actara 10X	68.18	91.32	2.40	1.53	4.74
	Actara 20X	55.44	94.09	1.72	1.39	2.79
	Verita 0.15%	43.42	95.62	2.03	0.81	1.52
	Verita 10X	68.70	90.27	3.05	1.55	5.12
	Verita 20X	62.47	90.34	2.72	3.47	3.47
Capsicum annuum	Control	75.40	94.50	2.50	1.30	1.70
	Actara 0.02%	58.20	88.90	4.90	4.40	1.80
	Actara 10X	61.30	86.60	4.70	5.80	2.90
	Actara 20X	66.20	87.30	5.60	5.30	1.80
	Verita 0.15%	63.10	89.50	5.30	4.60	0.60
	Verita 10X	60.00	87.10	5.90	5.80	1.30
	Verita 20X	58.10	85.20	6.70	7.00	1.10

Table 3. Frequency of chromosomal aberrations in *Allium cepa* and *Capsicum annuum* treated with Actara and Verita, %

Sample		Cells with micronuclei		Cells with anaphase and telophase bridges		Cells with chromosomal fragments and lagging chromosomes		Total cells with aberrations	
		1	2	1	2	1	2	1	2
Allium cepa	Control	0.41	0.51	0.32	0.40	0.13	0.16	0.86	1.07
	Actara 0.02%	0.27	0.38	1.23	1.71	0.23	0.32	1.73	2.41
	Actara 10x	0.32	0.47	0.68	1.00	0.63	0.94	1.63	2.41
	Actara 20x	0.14	0.25	0.41	0.74	0.54	0.98	1.09	1.97
	Verita 0.15%	0.26	0.61	0.13	0.30	0.09	0.20	0.48	1.11
	Verita 10x	0.00	0.00	0.76	1.10	0.31	0.45	1.07	1.55
	Verita 20x	0.71	1.13	0.85	1.36	0.05	0.07	1.61	2.56
Capsicum annuum	Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Actara 0.02%	0.52	0.89	0.17	0.30	0.13	0.22	0.82	1.41
	Actara 10x	0.28	0.46	0.09	0.15	0.14	0.23	0.51	0.84
	Actara 20x	0.20	0.30	0.05	0.08	0.10	0.15	0.35	1.25
	Verita 0.15%	0.18	0.29	0.00	0.00	0.00	0.00	0.18	0.29
	Verita 10x	0.04	0.07	0.04	0.07	0.00	0.00	0.08	0.14
	Verita 20x	0.04	0.07	0.12	0.21	0.00	0.00	0.16	0.28

1 – towards the total number of cells; 2 – towards mitotic cells

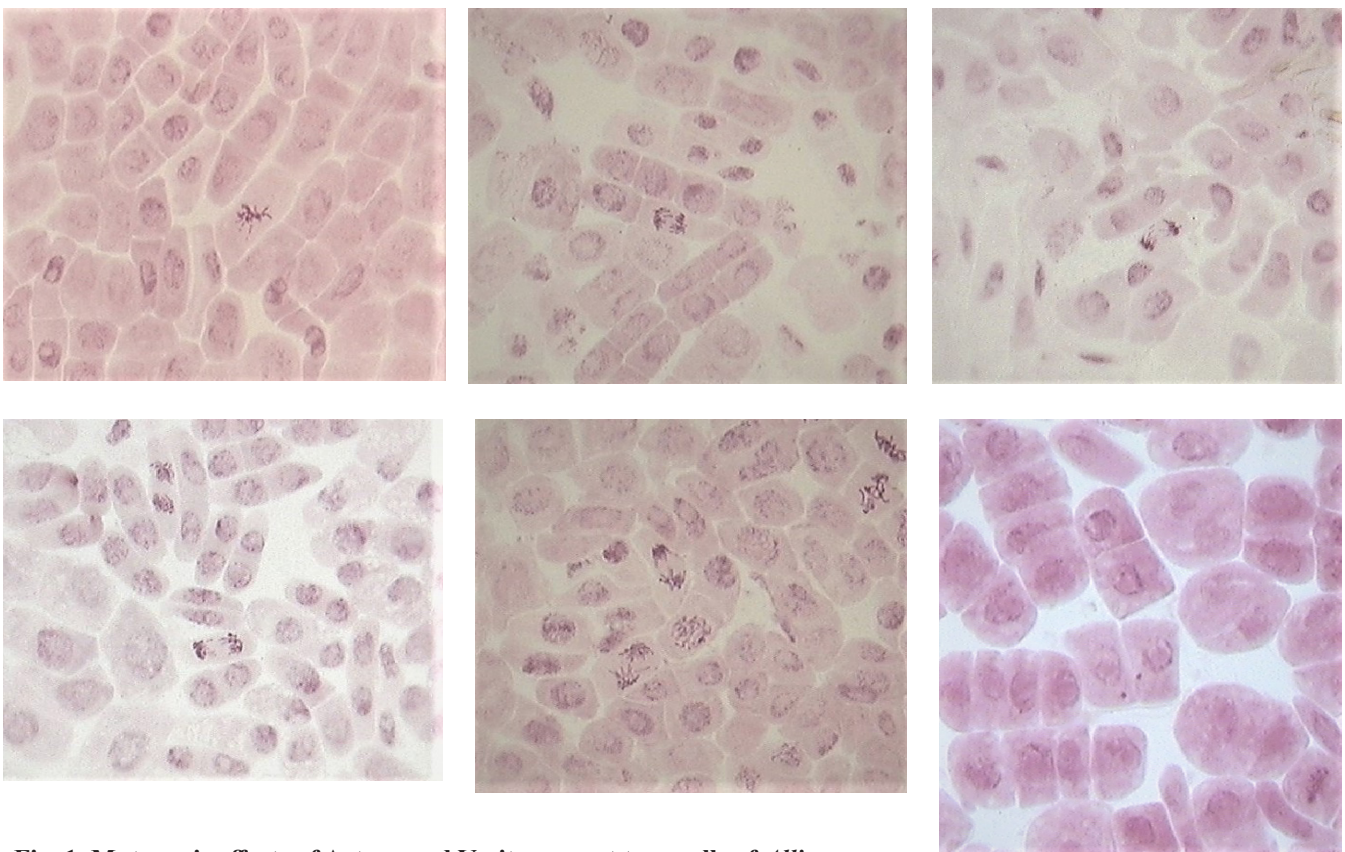

Fig. 1. Mutagenic effects of Actara and Verita on root type cells of *Allium cepa* and *Capsicum annuum* – anaphase/telophase bridges, lagging acentric chromosomes and chromosomal fragments, and micronuclei (400x)

Fisun & Rasgele, 2009; Asita & Mokhobo, 2013; Mesi & Kopliku, 2013; Yekeen & Adeboye, 2013; Sinha & Kumar, 2014; Liman et al., 2015).

Lower values of mitotic indices (IM) in the treated variants versus control showed that the pesticides may possess a cytotoxic effect. According to Asita and Matebesi (2010), the pesticide concentration could be accepted as cytotoxic when IM of treated cells is ≤ ½ of the control IM. In this aspect, our results do not indicate such big differences. At the same time, the cytostatic effects of both tested pesticides Actara and Verita were observed (Verita 0.15% in onion and lowest concentration in pepper; Actara 0.02% in pepper, Table 2).

The data concerning the frequency of chromosomal aberrations in the *Allium cepa* and *Capsicum annuum* root meristems (control and experimental samples treated with pesticides) are presented in Table 3. The following types of chromosomal aberrations were observed in the analyzed cells: anaphase (telophase) bridges, lagging chromosomes and/or chromosome fragments, and micronuclei (Figure 1). In the onion control sample, chromosomal aberrations were observed also, probably due to auto-mutagenesis. In the control sample of pepper, chromosomal disorders were not detected, indicating a lack of auto-mutagenic processes.

Higher values of total frequency of chromosomal aberrations, as well as the frequency of aberrations in dividing cells, were recorded in *Allium cepa* after treatment with pesticide Actara in the recommended concentration (0.02%). The highest percentages of cells with induced mutagenesis were found in onion (1.73% of cells analyzed, and 2.41% of dividing cells). In pepper, higher values of the total chromosome aberration frequency, as well as the frequency of chromosomal aberrations among fission cells, were found after treatment with Actara (0.02%). In tested species, onion and pepper, parallel with decreasing of concentrations of Actara solutions, there was observed a tendency toward decreasing the total number of cells with chromosomal abnormalities. The recommended concentration (0.02%) of Actara possesses pronounced mutagenic effect. In the onion cells, this insecticide induces the highest number of cells with anaphase bridges; in pepper cells – the highest number of cells with micronuclei.

In the samples that were treated with Verita chromosomal abnormalities were also found. In onions, higher percentages of cells with aberrations were recorded at the lowest fungicide concentration (1.61% and 2.56%), and in pepper – in the samples treated with the recommended concentration (0.18% and 0.29%) as well as with the lowest one (0.16% and 0.28%).

An analogous mutagenic effect of pesticides has been published by other authors (Dimitrov et al., 2006; Singh et al., 2007; Asita & Matebesi, 2010; Topcu et al., 2013; Martínez-Valenzuela et al., 2017). Similar to the results in the present study, a positive correlation between the percentage of chromosomal aberrations and the concentration of the tested pesticide solutions was found by other authors also (Rufus et al., 2000; Aydemir et al., 2008; Fisun & Rasgele, 2009; Mesi & Kopliku, 2013; Topcu et al., 2013).

Conclusion

The fungicide Verita and the insecticide Actara have a mitosis-depressive action on the onion (*Allium cepa* L.) and sweet pepper (*Capsicum annuum* L.) root meristems. The Verita solution 0.15% that is recommended for practical application significantly reduces the pepper seeds' germination. Concerning *Capsicum annuum* L. both pesticides have genotoxic effects in the concentrations used in practice as well as in the lower concentrations. The results show that even minimal residual amounts of pesticides could have a mutagenic effect and damage plant chromosomes.

References

Alavanja, M. C., Hoppin, J. A. & Kamel, F. (2004). Health effects of chronic pesticide exposure: cancer and neurotoxicity. *Annual Review of Public Health*, *25*, 155–197.

Ali, D., Nagpure, N. S., Kumar, S., Kumar, R. & Kushwaha, B. (2008). Genotoxicity assessment of acute exposure of chlorpyrifos to freshwater fish *Channa punctatus* (Bloch) using micronucleus assay and alkaline single-cell gel electrophoresis. *Chemosphere*, *71(10),* 1823-1831.

Ansari, R. A., Kaur, M., Ahmad, F., Rahman, S., Rashid, H., Islam, F. & Raisuddin, S. (2009). Genotoxic and oxidative stress-inducing effects of deltamethrin in the erythrocytes of a fresh water biomarker fish species, Channa punctata Bloch. *Environnmental Toxicology*, *24(5),* 429–436.

Asita, A. O. & Matebesi, L.P. (2010). Genotoxicity of hormoban and seven other pesticides to onion root tip meristematic cells. *African Journal of Biotechnology*, *9(27),* 4225–4232.

Asita, A. O. & Mokhobo, M. M. (2013). Clastogenic and cytotoxic effects of four pesticides used to control insect pests of stored products on root meristems of *Allium cepa*. *Environment and Natural Resources Research*, *3(2),* 133-145.

Aydemir, N., Çelikler, S., Summak, S., Yılmaz, D. & Özer, Ö. (2008). Evaluation of clastogenicity of 4, 6-Dinitro-o-cresol (DNOC) in *Allium* root tip test. *Journal of Biological and Environmental Sciences*, *2(5),* 59-63.

Dimitrov, B. D., Gadeva, P. G., Benova, D. K. & Bineva, M. V. (2006). Comparative genotoxicity of the herbicides Roundup, Stomp and Reglone in plant and mammalian test systems. *Mutagenesis*, *21(6),* 375–382.

Dubey, P., Mishra, A. K., Shukla, P. & Singh, A. K. (2015). Differential sensitivity of barley (*Hordeum vulgare* L.) to chlorpyrifos and propiconazole: Morphology, cytogenetic assay and photosynthetic pigments. *Pesticide Biochemistry and Physiol-*

ogy, *124*, 29-36.

Fetoui, H., Feki, A., Salah, G. B., Kamoun, H., Fakhfakh, F., & Gdoura, R. (2015). Exposure to lambda-cyhalothrin, a synthetic pyrethroid, increases reactive oxygen species production and induces genotoxicity in rat peripheral blood. *Toxicology and Industrial Health*, *31(5),* 433-441.

Fisun, K. & Rasgele, P. (2009). Genotoxic effects of Raxil on root tips and anthers of *Allium cepa* L. *Caryologia*, *62(1),* 1-9.

Gill, R. & Shaukat, S. (2000). Genotoxic effects of Captan fungicide on root meristems of *Allium cepa* L. *in vivo*. *Pakistan Journal of Biological Sciences*, *3(1),* 114-117.

Liman, R., Ciğerci, I. H. & Öztürk**, N. S.** (2015). Determination of genotoxic effects of Imazethapyr herbicide in *Allium cepa* root cells by mitotic activity, chromosome aberration, and comet assay. *Pesticide Biochemistry and Physiology*, *118*, 38-42.

Martinez-Valenzuela, C., Waliszewski, S. M., Amador-Muñoz, O., Meza, E., Calderón-Segura, M. E., Zenteno, E., Huichapan-Martinez, J., Caba, M., Félix**-Gastélum, R. & Longoria-Espinoza, R.** (2017). Aerial pesticide application causes DNA damage in pilots from Sinaloa, Mexico. *Environmental Science and Pollution Research International*, *24(3),* 2412–2420.

Mesi, A. & Kopliku, D. (2013). Cytotoxic and genotoxic potency screening of two pesticides on *Allium cepa* L. *Procedia Technology*, *8*, 19–26.

Omari, Y. I. (2011). Micronucleus analysis and mitotic index in a Jordanian population exposed to pesticides of organophosphate: malathion and chlorpyrifos. *Caryologia*, *64(2),* 173-178.

Ozakca, D. U. & Silah, H. (2013). Genotoxicity effects of Flusilazole on the somatic cells of *Allium cepa*. *Pesticide Biochemistry and Physiology*, *107(1),* 38-43.

Rufus, S., Gill, A. & Shahid Shaucat, S. (2000). Genotoxic effect of captan fungicide on root meristems of *Allium cepa* L. *in vivo*. *Pakistan Journal of Biological Science*, *3(1),* 114-117.

Singh, P., Srivastava, A. & Singh, A. (2007). Comparative sensitivity of barley (*Hordeum vulgare* L.) to insecticide and fungicide on different stages of cell cycle. *Pesticide Biochemistry and Physiology*, *89*, 216–219.

Sinha, V. S. & Kumar, N. (2014). Assessment of Mito-inhibitory and genotoxic effects of two organophosphate pesticides in the root tip cells of *Allium cepa* L. *Annals of Plant Sciences*, *3(5),* 699-703.

Staykova, T., Ivanova, E. & Valcheva, I. (2005). Cytogenetic effect of heavy-metal and cyanide in contaminated waters from the region for of southwest Bulgaria. *Journal of Cell and Molecular Bioogy, 4,* 41-46.

Stoyanov, I., Vasileva, P., Popova, T., Staykova, T. & Ivanova, E. (2013). Toxic and mutagenic effect of Verita WG on *Pisum sativum* test-system in vivo. In: *Jubilee National Scientific Conference with International Participation "Traditions, Directions, Challenges" Smolyan, October 19th–21st, 2012.* Natural Science, Mathematics and Informatics (Natural and Agricultural Sciences. Medicine), *1(2),* 37-45.

Topcu, S., Yılmaz, F., Ergene, A. & Tan, S. (2013). Genotoxic effects of Dursban 4 in *Allium cepa*. *Hacettepe Journal of Biology & Chemistry*, *41(1),* 21-27.

Van Der Werf, H. (1996). Assessing the impact on the environment. *Agriculture, Ecosystems and Environment*, *60*, 81–96.

Wang, Q., Xia, X., Deng, X., Li, N., Wu, D., Zhang, L., Yang, C., Tao, F. & Zhou, J. (2016). Lambda-cyhalothrin disrupts the up-regulation effect of 17β-estradiol on post-synaptic density 95 protein expression via estrogen receptor α-dependent Akt pathway. *Journal of Environmental Sciences*, *41*, 252-260.

Yekeen, T. A. & Adeboye, M. K. (2013). Cytogenotoxic effects of cypermethrin, deltamethrin, lambdacyhalothrin and endosulfan pesticides on *Allium cepa* root cells. *African Journal of Biotechnology*, *12(41),* 6000-6006.

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