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RESIDUES OF ORGANOPHOSPHORUS PESTICIDES IN APPLES

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***Abstract:** Apples have a significant positive impact on human health. In the market, the apple supply lasts all year round and this is made possible by cultivating different types of apples. In the Republic of Macedonia, apples are mostly cultivated in the Prespa Region. During the process of cultivation there are used pesticides which belong to different chemical groups, such as organophosphate, organochlorine, pyrethroids, carbamates, dithiocarbamates etc. The purpose of this research was to determine the residue analysis of organophosphorus insecticides in apples: chlorpyrifos, dimethoate and omethoat. There were taken samples of apples from two different locations, Drmeni and Jankovec from Prespa Region. The processes of extraction/separation and purification were done using acetonitrile and dispersive SPE-QuEChERS method and for their analysis were applied UPLC-TQ/MS. The concentration of residues of insecticides in apples from Jankovec was in the range of 0.01-0.03 mg/kg while in apples from Drmeni the value was between 0.02-0.05 mg/kg. The results show that parts of the analyzed apples contain insecticides with a higher concentration than the maximum residue limit (MRL), therefore the apples are not safe for consumption. Contamination of fruits with pesticides residues in general is one of the most important aspects of the food quality assurance. In order to provide consumers with food that does not contain residues of pesticides above the MRL, it is necessary to reduce the use of pesticides and to increase the application of integrated protection of crops, as well as to monitor and control products from authorized institutions.*

***Keywords:** insecticides, apples, QuEChERS, UPLC-TQ/MS*

INTRODUCTION

Apples are one of the most important deciduous fruits belong to the family *Roseaceae* and the *Pomoideae* subfamily (Phipps, J. B., 1990). In the market, the apple supply lasts all year round and this is made possible by cultivating different types of apples. Their cultivation depends on the region, the climatic and geographical conditions, the amount of solar radiation, the dry summers and sharp winters, the rainy regions, etc., followed by the different resistance of varieties of apples to pests and diseases. The apple is a delicious fruit, easy to process, and is low on calories. It is a source of vitamins and minerals, including: vitamin A and C, calcium, phosphorus, iron, potassium, soluble and insoluble dietary fiber (Chen, M., Tao, L., McLean, J., & Lu., Ch., 2014). Apples have a significant positive impact on human health (Łozowicka, B., 2015).

Chemical measures for the protection of apples (use of pesticides) are the most effective in the eradication of pests and diseases (Balaz, J., Grahovac, M., Radunović, D., Iličić, R., & Krstić,

M., 2013). During the process of cultivation pesticides are used which belong to different chemical groups and have different mechanisms of action, such as organophosphates, organochlorines, pyrethroids, carbamates, dithiocarbamates, etc. (Jankuloska, V., Karov, I., Pavlovska, G., & Kalevska, T., 2017). Numerous countries are currently initiating programs to reduce pesticide usage in conventional agriculture (Sinha, N. S., Vasudev, K., & Rao, V. V. M., 2012). Organophosphorus pesticides (OPs) used mainly as insecticides, are esters of phosphoric acid with different substituents (Pagliuca, G., Gazzotti, T., Zironi, E., & Sticca, P., 2005). These substances act through inhibition of acetylcholinesterase and represent a risk to human health (Wesseling, C., Keifer, M., Ahlbom, A., Micconnell, R., Moon, J. D., Rosenstock, L., & Hogstedt, C., 2002).

Chlorpyrifos is an organophosphorus pesticide (insecticide) used to protect apples from insects that can cause significant harm in the production of apples (Jankuloska, V., Karov, I., Pavlovska, G., & Buzlevski, I., 2017). Dimethoate is a contact and systemic organophosphorus insecticide (acaricide) and its main toxicological endpoint in animals and humans is the inhibition of acetylcholinesterase (AChE) activity. Omethoate is an oxygen analogue metabolite of dimethoate and plays a dominant role in the toxicity of dimethoate for insects and mammals, i.e. it is a toxic metabolite of dimethoate. In acute exposures, this insecticide is considered highly toxic after oral intake and moderately toxic if it enters the body through the skin or is inhaled (Hayes, W. J., & Laws, E. R., 1990).

Pesticides that are allowed to be used as means of protection in the Republic of Macedonia are presented in the list of allowed active substances (Official Gazette of the Republic Macedonia., 105, 2013) and MRL for residue of pesticides are given on Rulebook on general requirements for food safety (Official Gazette of the Republic Macedonia., 156, 2013). A plant product or food should be destroyed or banned from use if it contains pesticide residue with a higher concentration than MRL (maximum residue limit). Pesticide residue in agricultural products and foods above the MRL that is legally tolerated is the result of inadequate application and application of pesticides in agricultural practice and storage (Bursić, V., Vukovic, G., Spirovic, B., Lazić, S., Pucarevic, M., & Zeremski, T., 2013). Therefore it is necessary to pay attention to withdrawal periods for each pesticide (Sadło, S., Szpyrka, E., Jaźwa, A., & Zawisłak, A., 2007). Although the application of pesticides is high, in the last years the integral protection of apples has gained significance. This type of protection involves the use of natural agents for suppression and use of selective insecticides, which preserve the environment, while simultaneously contributing to the production of health food safety food (Miletić, N., & Tamaš, N., 2009; Fenik, J., Tankiewicz, M., & Biziuk, M., 2011).

EXPOSITION

Sampling and analysis methods

Samples of apples were taken from two different locations, Drmeni and Jankovec from the Prespa Region. Apples of the Golden Delicious variety were analyzed.

In order to analyze pesticide residue it is necessary to prepare the sample and extract the residue, and then to analyze it by using separation techniques. Today, the most commonly used method for extracting pesticide residues is QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe). QuEChERS involves several simple analytical steps, which are easy to perform and are slightly sensitive to errors. Thus, a high yield of extraction of a number of analytes is ensured, and the final extract can be directly analyzed by gas or liquid chromatography (Stocska, J., & Biziuk, M., 2015). Following the new trends in the extraction of pesticide residues, the QuEChERS method was applied in this study (Anastassiades, M., Lehotay, S. J., Stajnbaher, D., & Schenck, F. J., 2003), according to the MKC EN 15662:2011 standard (Jankuloska, V., Karov, I., & Pavlovska, G., 2018).

Pesticides were analyzed with ultra-high-performance liquid chromatography, a consequence of HPLC whose basic principle is that, as the size of the particles (2 µm) in the column decreases, efficiency and resolution increase (Patil, V. P., Tathe, R. D., Devdhe, S. J., Angadi, S. S., & Kale, S. H., 2011; Kumar, P., Singh, S. P., Shrikant, K., & Madhukar, D., 2011). For the analysis of pesticide residues by UPLC-TQ/MS was used Agilent UPLC 1290, detector DAD VL Agilent 1260 G1315D (Waldbronn, Germany), Agilent triple quadrupole LC/MS detector 6420 (Agilent

Technologies, Santa Clara, California, USA). The temperature of the column was 35°C and the flow was 0.4 ml/min. The volume of injection is 0,7µl.

The name of the chemical, the withholding period (WHP) and retention time of the analyzed organophosphorus pesticides is given in Table 1.

Table 1. Chemical name and withholding period (WHP) of a group of pesticides

| Pesticide | Chemical name | WHP (days) | Retention time (min) |
|--------------|---|------------|----------------------|
| chlorpyrifos | O, O-diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothioate | 28 | 13,622 |
| dimethoate | 2-dimethoxyphosphinothioylthio-N-methylacetamide | 7 | 4,310 |
| omethoate | 2-dimethoxyphosphinoylthio-N-methylacetamide | 21 | 2,70 |

RESULTS AND DISCUSSION

The results for the analyzed residues of pesticides in Golden Delicious apples, grown at the location of Drmeni and Jankovec are presented in Figure 1.

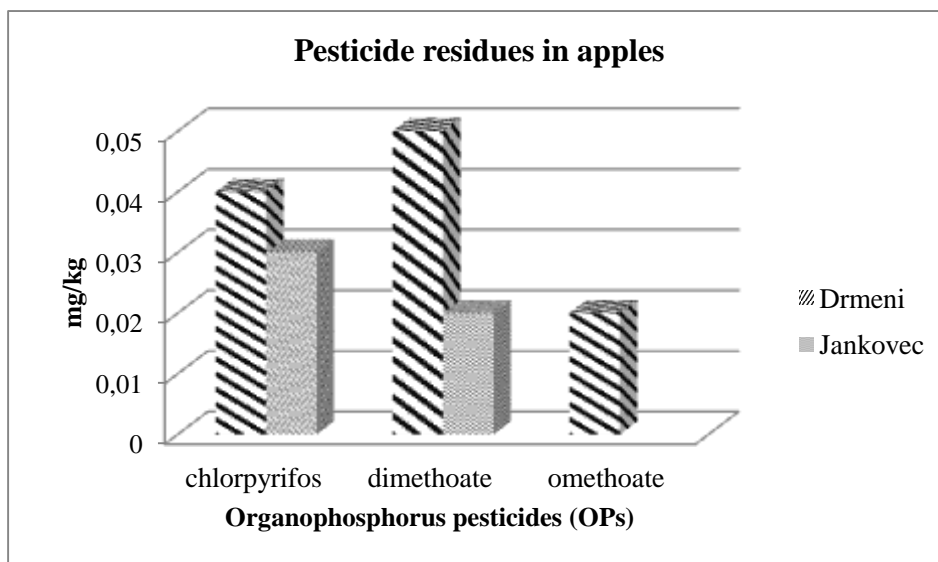


Fig. 1. Residues of organophosphorus pesticides (OP_s) in Golden delicious from Drmeni and Jankovec

The concentration of residues of insecticides in apples from Jankovec was in the range of 0.02-0.03 mg/kg, while in apples from Drmeni the value was between 0.02-0.05 mg/kg. It is noticeable that chlorpyrifos and dimethoate are present in the apples from both locations while the omethoate is detected only in the apples from the Drmeni location (Fig.1).

A side by side comparison of the pesticide residue in apples from Jankovec and their MRL is given in Figure 2.

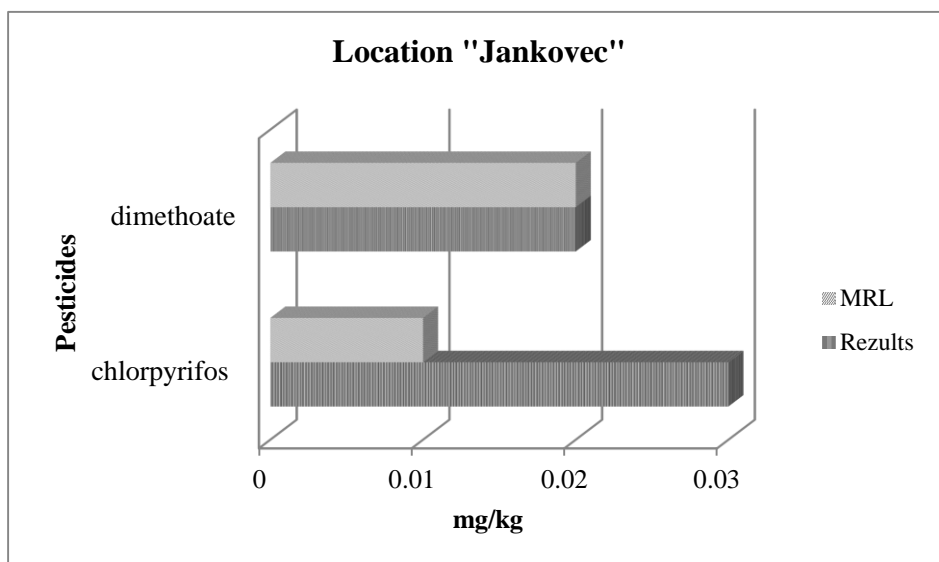


Fig. 2. Comparison of the pesticide residues in apples from the location of Jankovec with MRL

As can be seen from the results shown, chlorpyrifos residues were measured at 0.03 mg/kg, and its MRL is 0.01 mg/kg. It can be concluded that chlorpyrifos residue is three times higher than the MRL, while the insecticide dimethoate residue (0.02 mg/kg) is equal to the MRL (0.02 mg/kg). From the obtained results, it can be concluded that in relation to the insecticide chlorpyrifos, apples of the Golden Delicious variety from Jankovec, are not safe to eat.

A comparison of the concentration of detected pesticide residues in the apple from the location of Drmeni and MRL is given in Figure 3.

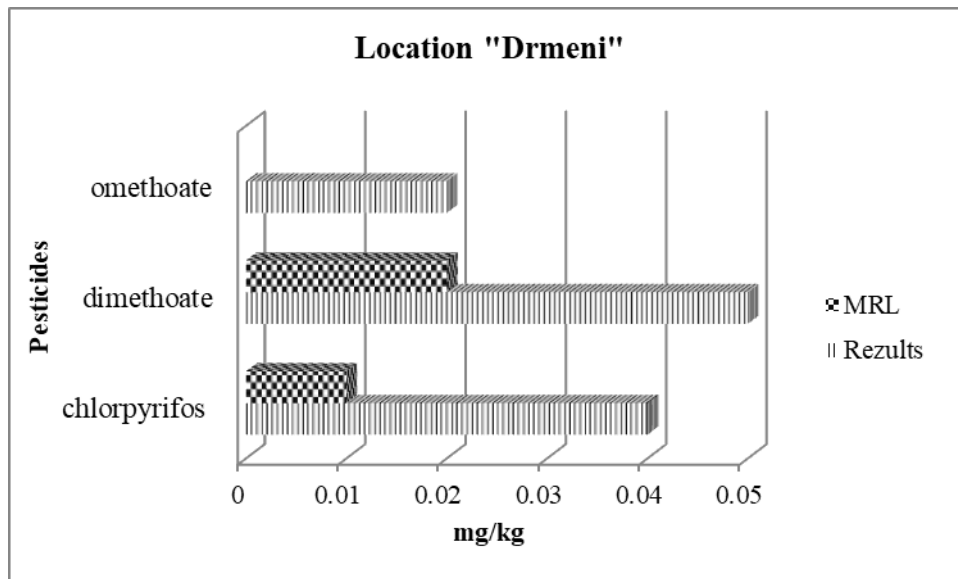


Fig. 3. Comparison of the pesticide residues in apples from the location of Drmeni with MRL

In apples from the location of Drmeni, the residue of insecticides chlorpyrifos, dimethoate and omethoate is higher than the tolerable MRL. The chlorpyrifos residue level is 0.04 mg/kg and the MRL is 0.01 mg/kg, a level four times higher than the MRL. Dimethoate residue level was detected with 0.05 mg/kg and its MRL is 0.02 mg/kg, which means that its level is 2.5 times higher than the MRL. No residue limit for omethoate is provided in the Rulebook for Maximum Residue Limit. But the apples from this location have been shown to contain a residue of 0.02 mg/kg. As a result of the above, it can be concluded that the Golden Delicious variety of apples from the Drmeni location are not safe for consumption, having been found to contain chlorpyrifos, dimethoate and omethoate residue above the MRL.

CONCLUSION

The results show that parts of the analyzed apples contain insecticides residue higher than the maximum residue limit (MRL), and therefore the apples are not safe for consumption. Contamination of fruits with pesticides residues in general is one of the most important aspects of the food quality assurance. In order to provide consumers with food that does not contain residues of pesticides above the MRL, it is necessary to reduce the use of pesticides and to increase the application of integrated protection of crops, as well as to monitor and control products from authorized institutions.

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