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DETERMINATION OF FENBUCONAZOLE IN APPLES (GOLDEN DELICIOUS) FROM DIFFERENT LOCATION

Teaching Assistant Vezirka Jankuloska, M.Sc,

Faculty of Technology and Technical Sciences, Veles,
University "St. Kliment Ohridski", Bitola, R. Macedonia
Phone: +389 75 421 592
E-mail: vezirka.jankuloska@gmail.com

Prof. Ilija Karov, PhD

Faculty of Agriculture, Shtip
University "Goce Delcev", Shtip, R. Macedonia
E-mail: ilija.karov@ugd.edu.mk

Associate Prof. Gorica Pavlovska, PhD

Faculty of Technology and Technical Sciences, Veles,
University "St. Kliment Ohridski", Bitola, R. Macedonia
E-mail: gorica.pavlovska@gmail.com

Assistant Prof. Tatjana Kalevska, PhD

Faculty of Technology and Technical Sciences, Veles,
University "St. Kliment Ohridski", Bitola, R. Macedonia
E-mail: tkalevska@gmail.com

***Abstract:** Fenbuconazole is a triazole fungicide that works systemically to prevent the growth of fungi by interrupting their normal growth cycle. Fenbuconazole is used to control powdery mildew, leaf spots and blotches, rusts, smuts, root/stem rots and fruit scab. Fenbuconazole is a systemic fungicide, meaning it is absorbed and translocated throughout the plant. It interferes with fungal sterol production, thereby slowing or stopping fungal growth. Fenbuconazole is primarily used as a preventative fungicide. The aim of our research is to determine the presence of fenbuconazole in apples (Golden Delicious) grown in two different locations. The residues from fenbuconazole in apples were extracted with QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) technique. The fungicide analysis is performed with LC-MC/MC. The concentration of fenbuconazole is analyzed in four development phases of the apples and it is in range from 0.01-0.07 mg / kg. In order to see whether apples are safe for consumption, a comparison of the obtained concentration with the maximum residue limits (MRL) for fenbuconazole was made. The obtained results show that there is a significant difference in the presence and use of fenbuconazole as a protective agent, in the same variety of apples of a different geographical location.*

***Keywords:** fenbuconazole, pesticides, apple, Golden Delicious, maximum residue level (MRL)*

INTRODUCTION

In the present agricultural practice, pesticides provide an unquestionable benefit for crop protection (Dobrinas, S., Stancium, G., Soceanu, A., & Culea, A., 2011). Pesticides are substances of natural or synthetic origin used as biocides to combat pests of crops, livestock, domestic animals and his environment (Morales, A., Ruiz, I., Olivia, J., & Barba, A., 2011). Pesticides are used in the production of fruits, vegetables and cereals (Poulsen, E.M., Naef, A., Gasser, S., Christen, D., Rasmussen, H. P., (2009). During production, various diseases and pests attack apples. To protect them, various measures are applied. Chemicals and pesticide treatments are most used. In the production of apples, fungicides are used to protect the fruits from a wide range of pests and fungi, such as: powdery mildew, leaf spots and blotches, rusts, smuts, root/stem rots, and fruit scab.

Fenbuconazole or 4-(4-chlorophenyl)-2-phenyl-2-(1,2,4-triazol-1-ylmethyl)butanenitrile is a preventive fungicide and is used to control powdery mildew, leaf spots and blotches, rusts, smuts, root/stem rots, and fruit scab (The Dow Chemical Company, 2011).

The traces of pesticides left in treated products are called "residues". A maximum residue level (MRL) is the highest level of a pesticide residue that is legally tolerated in or on food when pesticides are applied correctly (Good Agricultural Practice) (Official Gazette of the Republic Macedonia, 2013). The presence of these residues and their metabolites may be a potential hazard to human health therefore a priority goal is to determine their presence in food in order to avoid the possible risk to human health.

The number of pesticides registered for use increases every year and many pesticides that have been forbidden because of health reasons are still being used illegally. Moreover, the introduction of new pesticides in the field of residue analysis also caused the laboratories involved in the analysis to face a more challenging task. This led to the development of many multiresidue methods by various researchers (Fillion, J., Sauv e, F., & Selwyn, J., 2000; Aysal, P., Ambrus,  ., Lehotay, S. J., & Cannavan, A., 2007; Hanim, N. H., Khalil, B., & Huat, G. T., 2012).

In order to analyze the pesticide residues it is necessary to prepare the sample and extract the residues and analyze them with an analytical or instrumental method.

The QuEChERS method uses acetonitrile (the best characteristic for extraction with a wide range of pesticides with at least coextractives), acetic acid (pH adjustment), buffers (preventing the degradation of pH-sensitive analyte) and NaCl (reduces polar interference). MgSO₄ is used as clean-up reagents for removing interferences, separating water from the organic phase (Anastassiades, M., Lehotay, S. J.,  tajnbaher, D., & Schenck, F. J., 2003; Bursic, V., Vukovic, G., Bojana Spirovic, Lazi c, S., Pucarevic, M., & Zeremski, T. 2013). The QuEChERS method comprises three phases: liquid micro extraction, solid phase purification and analysis of LC/MS or GC/MS.

In many cases, gas chromatography is a technique used to analyze pesticide residues in fruits and vegetables. But in recent years the trend has been the use of polar pesticides that have low persistency and toxicity in contrast to non-polar components. These polar pesticides are less stable for analysis with gas chromatography and therefore other alternatives are required. Therefore, LC-MS liquid chromatography has become a powerful technique for analyzing low-stability pesticide residues or thermally unstable foods and food samples (Soler, C., Ma es J., & Yolanda Pic , Y., 2008; Morales, A., Ruiz, I., Olivia, J., & Barba, A., 2011).

EXPOSITION

Determination of fenbuconazole concentrations in apples from location Evla

Apples of the Golden Delicious variety were grown in two different concentrations (Evla and Kriveni) from Resen, Republic of Macedonia. The concentration of fenbuconazole is determined by liquid mass chromatography and mass detector (LC-MS / MS) according to EN 15662:2011 method.

The concentration of fenbuconazole in apples from Evla is shown in Figure 1.

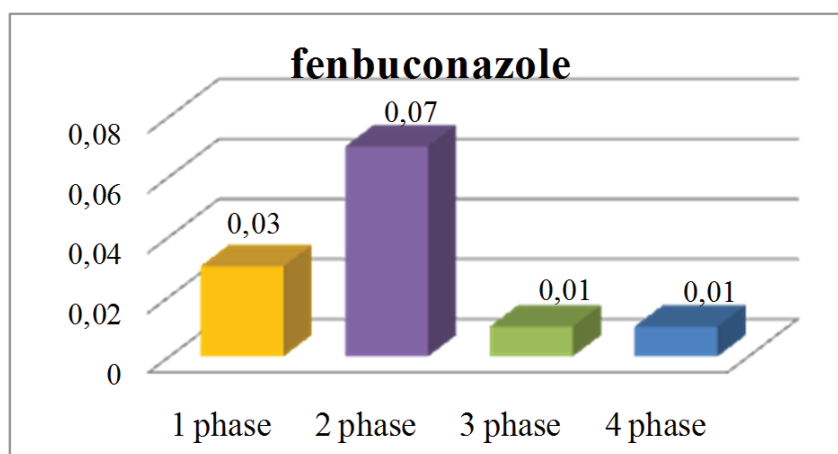


Fig. 1. Concentration of fenbuconazole in Golden Delicious from Evla

Figure 1 shows that this fungicide is present in all four phases of development of the apple, starting with the first phase (apple hazelnut size), the second phase (apple with the size of a nut), the third phase (apple during maturation) and fourth phase (apples during harvesting). The concentration of fenbuconazole is in rang from 0.01 to 0.07 mg/kg.

Determination of fenbuconazole concentrations in apples from location Kriveni

In apples from the Kriveni location, fenbuconazole is not detected in any developmental phase of the apples or is present in a concentration that is below the detection limit (0.01 mg / kg) (Table 1).

Table 1. Fenbuconazole in apples from location Kriveni

Apples development phase	Obtained results	LOD (limit of detection)
1 phase	<LOD	0,01 mg/kg
2 phase	<LOD	0,01 mg/kg
3 phase	<LOD	0,01 mg/kg
4 phase	<LOD	0,01 mg/kg

Comparison of obtained results with maximum residue level (MRL)

It is known that certain pesticides have an impact on health, for this reason the European Commission has adopted a list of maximum residues limits (MRL) for residues from pesticides used in the production of food and feed regulated by Regulation EC 396/2005 (Official Journal of the the European Union, 2005).The maximum residues limits (MRL) of fenbuconazole in apples is 0.5 mg / kg.

A comparison of the concentration of fenbuconazole in the fourth phase (apples during harvest) was made with the maximum residue levels (MRL) in order to see if apples are safe for consumption (Figure 2).

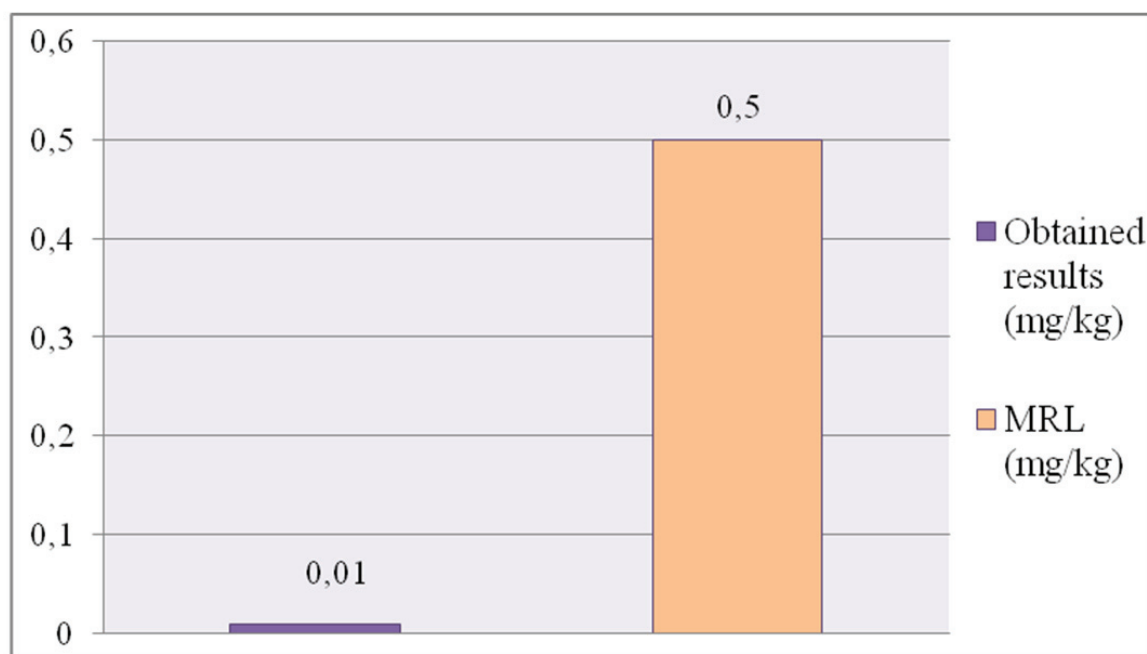


Fig. 2. Concentration of fenbuconazole in Golden Delicious from Evla and compared with MRL

From Figure 2 we can conclude that the concentration of fenbuconazole in Gold Delicious form Evla is 0.01 mg/kg and is 50 times less than the maximum residues levels (MRL) (0.5 mg/kg).

CONCLUSION

This study was designed to investigate the presence and the residues of fenbuconazole in apples (Golden Delicious) from two different locations (Evla and Kriveni) in Resen, R. Macedonia. The analysis was performed with LC/MS-MS and the extraction of the residues was made with the QuEChERS method. In apples from the location of Evla, the concentrations of fenbuconazole are from 0.01 to 0.07 mg/kg. In apples from the location Kriveni, this pesticide is not present or is at a concentration below the limit of detection. After comparisons with MRL for fenbuconazole, from the obtained data it was observed that the concentration of fenbuconazole is lower than MRLs imposed in the last EU regulation and the apples are safe for consumption. The results show that the geographical location of growing apples has an important role in the use of this pesticide which is associated with its representation in the same variety of apples.

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