

Dissipation rate of acetamiprid in sweet cherries

Sanja Lazić¹, Dragana Šunjka^{1*}, Srđan Panić¹, Dušanka Indić¹, Nada Grahovac²,
Valéria Guzsvány³ and Pavle Jovanov⁴

¹ *University of Novi Sad, Faculty of Agriculture, Department of Environmental
and Plant Protection*

² *Institute of Field and Vegetable Crops, Novi Sad*

³ *University of Novi Sad, Faculty of Sciences*

⁴ *University of Novi Sad, Institute of Food Technology*

**(draganas@polj.uns.ac.rs)*

Received: November 7, 2013

Accepted: December 27, 2013

SUMMARY

Degradation of acetamiprid in sweet cherry samples was evaluated at several intervals from the product application until the end of the pre-harvest interval. An orchard of sweet cherries located at Stepanovićevo village near Novi Sad was used in this study. Acetamiprid was applied according to the manufacturer's recommendation for protecting sweet cherries from their most important pests. Sweet cherry fruit samples were collected at eight intervals: immediately after acetamiprid application and 2, 4, 6, 8, 10, 12 and 14 days after application. The extraction of acetamiprid from sweet cherry samples was performed using a QuEChERS-based method. Determination was carried out using an HPLC-UV diode array detection system (Agilent 1100, United States) with an Agilent Zorbax Eclipse C18 column (50 mm × 4.6 mm internal diameter, 1.8 μm particle size). The method was subjected to a thorough validation procedure. The recovery data were obtained by spiking blank sweet cherry samples at three concentration levels (0.1–0.3 mg/kg), yielding 85.4% average recovery. Precision values expressed as relative standard deviation (RSD) were below 1.61% for the intraday precision. Acetamiprid showed linear calibrations from 0.05 to 2.5 μg/ml with correlation coefficient (R^2) of 0.995%. The limit of detection and limit of quantification were found to be 5 μg/kg and 14 μg/kg, respectively. The validated method was applied in the analysis of acetamiprid in sweet cherry samples. During the study period, the concentration of acetamiprid decreased from 0.529 mg/kg to 0.111 mg/kg. The content of acetamiprid in sweet cherry samples at the end of the pre-harvest interval was below the maximum permissible level specified by the Serbian and EU MRLs.

Keywords: Acetamiprid; Sweet cherries; Dissipation; Residues

INTRODUCTION

The use of pesticides in contemporary agricultural production helps to increase yields and improve the quality of products. However, their inadequate usage leads to accumulation of their residues in the environment and agricultural products. This is especially important for fruits and vegetables that are mostly consumed fresh. One of such fruit species is the sweet cherry as only 15% of its overall production is planned for processing (Commission of the European Communities, 2006).

Sweet cherry (*Prunus avium* L.) is a highly valued fruit species owing to its pleasant taste, but also because of its nutritive value. It is a significant species with considerable potentials in terms of export to the international market. As the acreage of this crop is increasing worldwide, the producers are facing a challenge to their efforts to produce quality fruit despite the increasing pressures from harmful agents. One of the most important pests of the sweet cherry is the cherry fly (*Rhagoletis cerasi* L.). Its attack results in a reduced market value of fruits, and they additionally become susceptible to saprophytes and frequently to rot, and fall prematurely. The control of this pest is a great challenge in integrated as well as conventional systems of production, and both in terms of ecotoxicology and pesticide residues (Kovanci and Kovanci, 2006).

Its control is successfully performed by insecticides of the group of organophosphates. However, their application time and toxicity, and especially the short period of fruit ripening and the violation of its prescribed harvest waiting period create preconditions for residues of these compounds to occur in sweet cherry fruits. Good agricultural practice therefore requires that protection be performed with products that have shorter harvest waiting periods and more convenient ecotoxicological properties than the insecticides used at earlier times (Lazić et al., 2012).

Acetamiprid, a neonicotinoid insecticide, has been introduced as an alternative to organophosphate insecticides for control of major cherry pests (Table 1). It is an antagonist of the acetylcholine receptor in *postsynapses* of insects that has excellent systemic properties, relatively low toxicity to warm-blooded organisms and long lasting effect. The pre-harvest period for acetamiprid in sweet cherries is 14 days (Sekulić and Jeličić, 2013).

However, due to a growing use of insecticides from the family of neonicotinoids, their increased presence in the environment is evident. The European Commission has adopted a proposal for a two-year restriction on the use (as of 1 December 2013) of three pesticides belonging to the neonicotinoid family (clothianidin, imidacloprid and thiamethoxam) (Official website of the European Commission, 2013).

Besides its positive effects, acetamiprid also poses various health risks to consumers (Lazić et al., 2012a). Pesticide residues can be found even when products are applied in accordance with good agricultural practices. The European Union specified a maximum residue level (MRL) for acetamiprid in sweet cherries of 0.5 mg/kg [Commission Regulation (EU) No. 978/2011], while it is 1.5 mg/kg under the EU Reg. No. 500/2013. The maximum permissible level for acetamiprid in sweet cherries set by the Serbian legislation is 0.2 mg/kg (Pravilnik, 2010).

The dissipation rate of pesticides after application depends on many factors, including their chemical and photochemical degradation, climatic conditions, volatilization, cultivated species, formulation class and application method (Sur et al., 2000).

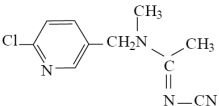
The aim of this study was to generate data showing the persistence and residue levels of acetamiprid in sweet cherry fruits under controlled conditions. The extraction of acetamiprid from sweet cherry samples was done using a QuEChERS-based method with insecticide determination and quantification performed by HPLC/DAD.

MATERIAL AND METHODS

Field experiment

The study was conducted in a sweet cherry orchard located at Stepanovićevo village near Novi Sad. In order to protect the sweet cherry crop from *Rhagoletis cerasi* L., a commercial formulation (SP) with 200 g/kg acetamiprid active ingredient was used. The insecticide was applied by a portable hand sprayer and the solution was prepared at the recommended concentration of 0.025%, according to the manufacturer's instructions.

Table 1. Physicochemical properties of acetamiprid (Tomlin, 2006)

Common name/ molecular formula/CAS No.	Chemical name (IUPAC)	Structural formula
Acetamiprid C ₁₀ H ₁₁ ClN ₄ (160430-64-8)	(E)-N1-[(6-chloro-3-pyridyl)methyl]-N ² -cyano-N ¹ -Methylacetamide	

Sampling procedure

Field sampling was carried out during the technological maturity of a medium late sweet cherry variety. Sweet cherry samples were collected at eight intervals: immediately after acetamiprid application, and 2, 4, 6, 8, 10, 12 and 14 days after application. The samples were collected from different heights. The weight of each laboratory sample of sweet cherries was approximately 500 g. The samples were packed in plastic bags, hand delivered to a laboratory freezer and stored at -20 °C. Each sample was represented by triplicates used for calculation of the mean values of acetamiprid residue levels.

Chemicals and solutions

A certified standard of acetamiprid (purity 98.1%) was purchased from Dr Ehrenstorfer (Augsburg, Germany). The extraction solvent acetonitrile (ACN), of a suitable grade (HPLC) for pesticide residue analysis, and CH₃COOH were purchased from J.T. Baker (Germany).

The dispersive SP extraction (Cat. No. 5982-5650) and clean-up (Cat. No. 5982-5056) kits for QuEChERS sample preparation were purchased as ready-to-use from Agilent Technologies (USA). The water was purified with a water purification system (TKA, Germany).

A stock solution of acetamiprid was prepared in acetonitrile at a concentration of 100 µg/ml and stored at -10 °C, in the dark. Calibration solutions for the HPLC analysis were prepared by further dilution with acetonitrile, achieving concentrations in a range from 0.05 to 2.5 µg/ml.

Validation of the analytical method

The extraction and determination procedures had been optimized in our previous study (Lazić et al., 2013). Insecticide determination and quantification were performed by HPLC with diode-array detection (Agilent 1100 Series LC system, United States) and Agilent Zorbax Eclipse C18 column (50 mm × 4.6 mm internal diameter, 1.8 µm particle size). The analysis was done under the conditions described below (Table 2).

Table 2. Conditions for HPLC/DAD analysis of acetamiprid

Mobile phase	ACN/ ultrapure water with 1.5% CH ₃ COOH
Mobile phase ratio	30/70
Column temperature	25 °C
Flow rate	1.000 ml/min
Wavelength	254 nm
Injected volume	2.5 µl

Under the selected conditions, the linearity of detector response was evaluated at a concentration range between 0.05 and 2.5 µg/ml using nine calibration solutions prepared in acetonitrile. Calculations were done using the peak areas and linear regression was used for the calibration curve. The linearity of calibration curves was expressed by regression equation and the correlation coefficient (R^2).

The limit of detection was determined at a signal-to-noise ratio of three, whereas the limit of quantification (LOQ) was determined by considering a signal-to-noise ratio of 10.

The accuracy and precision of the proposed method were evaluated by spiking blank sweet cherry samples to fulfill all the necessary requirements of *SANCO/12495/2011* – the Method Validation and Quality Control Procedures for Pesticide Residues Analyses in Food and Feed (EU Commission Health and Consumer Protection Directorate-General, 2011).

Precision was evaluated through the repeatability of acetamiprid determination. Repeatability was checked by injecting 2.5 µl of acetamiprid standard in the matrix (0.75 and 1.5 µg/ml, respectively) five times. The samples were analyzed on the same day, using the same instrument by the same operator, and repeatability was calculated as a relative standard deviation (RSD%).

The accuracy of the proposed method was evaluated as the mean recovery (%) at three spiking levels. According to the EU validation guideline for pesticide residues, the mean recovery values should be within the range of 70-120% at each spiking level with RSDs ≤ 20%.

Sample extraction

The extraction of acetamiprid from sweet cherry samples was performed using the QuEChERS-based method (Anastassiades et al., 2003). Each homogenized sweet cherry sample (10 g) was weighed into a polypropylene tube (50 ml volume), 10 ml volume of ACN was added as an extraction solvent and the tube was tightly capped and vigorously shaken for 1 min. A mix of buffered salts (1000 mg of sodium citrate, 500 mg of sodium hydrogen citrate sesquihydrate, 4000 mg magnesium sulphate and 1000 mg sodium chloride) from separate pouches was added to each tube and immediately mixed for 1 min. After that, the tube was centrifuged at 3000 rpm for 5 min (Sigma, Germany). An aliquot of 6 ml of the upper acetonitrile layer was transferred to each 15 ml centrifuge tube containing the sorbent, 150 mg of primary-secondary amine (PSA) and 900 mg of magnesium sulphate. The tubes were vigorously mixed

for 1 min and then centrifuged at 3000 rpm for 5 min. An aliquot of the final upper layer was filtered through a 0.45 μm membrane filter and transferred into an autosampler vial for HPLC/DAD analyses.

RESULTS AND DISCUSSION

Method validation

In some official methods, acetamiprid is determined by the GC/NPD (Ministry of Health and Welfare of Japan, 1997). However, GC determination can produce overestimated values of highly polar compounds such as acetamiprid because of the matrix-induced enhancement effect (Sasaki et al., 1998). For determination of

other neonicotinoids, such as imidacloprid, the high-performance liquid chromatography is already commonly used (Mandić et al., 2005). In this study, acetamiprid was determined by HPLC. HPLC/DAD chromatograms of acetamiprid standard in acetonitrile and in spiked sweet cheery samples at a concentration of 0.75 $\mu\text{g}/\text{ml}$ are shown in Figure 1. UV apex spectrums of acetamiprid in ACN and in matrix are illustrated in Figure 2.

In the studied range of mass concentrations of acetamiprid, a good linearity of detector response was achieved. The obtained values suggest that the increase in concentration of acetamiprid working solutions linearly followed the increase in peak area. The calibration curves were linear over the range with the correlation coefficient of 0.995.

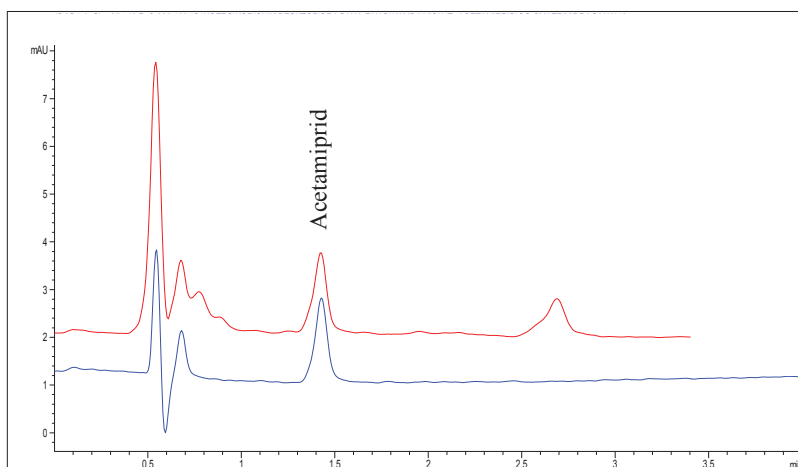


Figure 1. Comparison of HPLC/DAD chromatograms of acetamiprid in ACN (blue) and spiked sweet cherry (red) at a concentration of 0.75 $\mu\text{g}/\text{ml}$

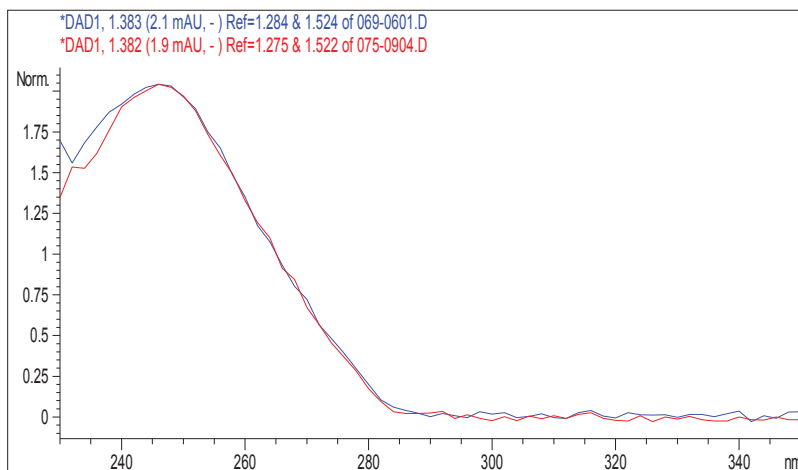


Figure 2. UV apex spectrum of acetamiprid in ACN and matrix

The precision of measurement of an analyte can be evaluated as repeatability or reproducibility. In this study, precision is expressed as repeatability. Precision was examined by analysing the same samples ($n=5$) at two different concentrations on the same day. The retention time of acetamiprid was 1.382 min. The RSD values were within the range of 0.06–0.25% for retention times and from 0.96 to 1.61% for peak area, fulfilling the mentioned criteria of $RSD \leq 20\%$ (EU Commission Health and Consumer Protection Directorate-General, 2011).

The calculated LOD, determined as an S/N ratio of three, and LOQ, determined by considering an S/N of 10 by using matrix-matched calibration curves, are 5 and 14 $\mu\text{g}/\text{kg}$, respectively. The presented LOQ is lower than the acetamiprid MRLs in sweet cherries set by the Serbian legislation and the European Commission.

The accuracy of the proposed method was evaluated as recovery, using blank samples spiked with the solution of acetamiprid insecticide at three levels (0.1, 0.2 and 0.3 mg/kg). A sweet cherry sample from a known locality without acetamiprid contamination was used as the blank sample. In this study acetamiprid was successfully extracted with acetonitrile, while in other studies neonicotinoid insecticides had been extracted with acetone and eluted from columns with dichloromethane (Tsumura et al., 1998, cit. Obana et al., 2002). Imidacloprid was extracted with acetone and transferred to a mixture of dichloromethane and petroleum ether to reduce interferences (Fernandez-Alba et al., 1996). The use of dichloromethane in these methods is not advisable because of environmental concerns (Obana et al., 2002).

The mean recovery achieved was 85.4% with an associated relative standard deviation (RSD) of 2.5%. The

average recoveries and RSD of the analyzed samples complied with the EU Commission Health and Consumer Protection Directorate-General (2011) criteria (70–120%), and were also used for method validation. Having in mind that the maximum permissible level of acetamiprid in sweet cherries is 0.2 mg/kg (Pravilnik, 2010), the method is sensitive enough for determination of that pesticide at concentrations well below the permissible level.

Acetamiprid residues in treated sweet cherry samples

The validated method was applied to analyse acetamiprid residues in sweet cherry samples, to which acetamiprid was applied under controlled conditions. The detected pesticide was selected by a respective retention time and UV spectra comparison with the reference standard. To obtain quantitative data, a method of external standard with calibration on nine levels was used.

Considering the importance of fresh fruits in a healthy diet, the concentration of acetamiprid residues in agricultural products should be monitored. The identification of acetamiprid in this study was based on its retention time and the spectrum was obtained by comparing it with the R_t standard of acetamiprid and its spectrum. No residues were detected on sweet cherry samples collected immediately before acetamiprid application (Figure 3).

Acetamiprid residues in sweet cherry samples after its application are presented in Figure 4. The maximum residue levels of acetamiprid were detected in the samples collected immediately after its application (0) with an average concentration of 0.529 mg/kg.

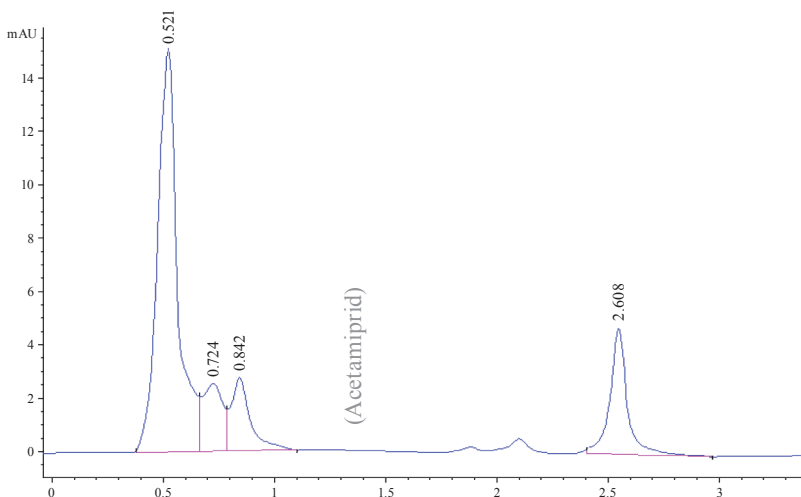


Figure 3. HPLC/DAD chromatogram of a blank uncontaminated sweet cherry sample

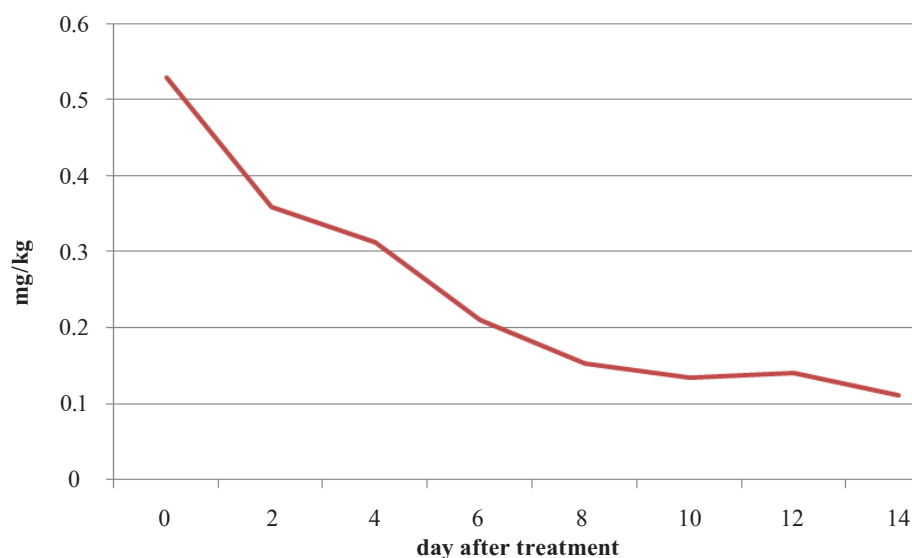


Figure 4. Acetamiprid residues in sweet cherry samples at various time intervals following its application

The samples collected on day 2 had a mean acetamiprid residue concentration of 0.359 mg/kg, which corresponds to 32% dissipation rate. The degradation of acetamiprid increased, and the content of acetamiprid was 0.313 mg/kg 4 days after treatment. The content of acetamiprid in sweet cherry samples collected 6 days after treatment was at the maximum permissible level in Serbia (0.209 mg/kg). Eight and 10 days after treatment, the concentrations of acetamiprid were 0.152 and 0.134, and the respective dissipation rates 71 and 75%. In sweet cherry samples collected on day 12, the content of acetamiprid residues was 0.139 mg/kg (74%), a slightly higher concentration than it was on day 10, which may be due to an error in handling and/or analysis of the sample (Park et al., 2011). The pre-harvest interval for acetamiprid in sweet cherries in Serbia is 14 days (Sekulić and Jeličić, 2013). At that time interval, the concentration of acetamiprid in our sweet cherries samples was 0.111 mg/kg, with a dissipation rate of 79%.

In this study, a method for determination of acetamiprid residues in sweet cherry samples was described. It is fast and simple, and can be used as a routine acetamiprid residue control procedure in a laboratory. Considering the obtained values of analytical parameters, the proposed method proved to be an efficient and sensitive method for determination of acetamiprid contents in cherries samples. The validated method was applied in an analysis of acetamiprid residues in sweet cherry samples after application at the recommended dose in accordance with good

agricultural practice. During the period of evaluation (0-14 days), acetamiprid concentrations decreased from 0.529 mg/kg to 0.111 mg/kg. After 6 days, the content of acetamiprid was at the Serbian MRL level (0.2 mg/kg).

ACKNOWLEDGEMENTS

The authors acknowledge the financial supports received from the Ministry of Education and Science, the Republic of Serbia, grant TR31038, and from the Secretariat for Science and Technological Development of Vojvodina Province, project "Development of chemical methods for analysis of the insecticide acetamiprid" grant 114-451-4418/2013-03.

REFERENCES

- Anastassiades, M., Lehotay, S.J., Stajnbaher, D., & Schenck, F.J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and 'dispersive solid-phase extraction' for the determination of pesticide residues in produce. *Journal of AOAC International*, 86(2), 412-431. pmid:12723926
- Commission of the European Communities. (2006). Annex to the report from the Commission to the Council and the European Parliament. Retrieved from http://ec.europa.eu/agriculture/publi/reports/fruitveg/softfruit/workdoc_en.pdf

COMMISSION REGULATION (EU) No. 978/2011 of 3 October 2011 amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for acetamiprid, biphenyl, captan, chlorantraniliprole, cyflufenamid, cymoxanil, dichlorprop-P, difenoconazole, dimethomorph, dithiocarbamates, epoxiconazole, ethephon, flutriafol, fluxapyroxad, isopyrazam, propamocarb, pyraclostrobin, pyrimethanil and spirotetramat in or on certain products. *Official Journal of the European Union*, (2011). L 258/12,

EU Commission Health and Consumer Protection Directorate-General. (2011). *Method validation and quality control procedures for pesticide residues analysis in food and feed (Document No. SANCO/12495/2011)*. Brussels: EU Commission.

Fernandez-Alba, A.R., Valverde, A., Agüera, A., Contreras, M., & Chiron, S. (1996). Determination of imidacloprid in vegetables by high-performance liquid chromatography with diode-array detection. *Journal of Chromatography A*, 721(1), 97-105. pmid:8653199. doi:10.1016/0021-9673(95)00764-4

Kovanci, O.B., & Kovanci, B. (2006). Reduced-risk management of *Rhagoletis cerasi* flies (host race Prunus) in combination with a preliminary phenological model. *Journal of Insect Science*, 6(34), 1-10. doi:10.1673/031.006.3401

Lazić, S., Šunjka, D., Grahovac, M., Vuković, S., & Gvozdenac, S. (2012). Primena i ostaci organofosfornih insekticida u trešnji i višnji. *Biljni lekar*, 40(1), 57-64.

Lazić, S., Šunjka, D., Grahovac, N., Guzsány, V., Bagi, F., & Budakov, D. (2012). Application of liquid chromatography with diode-array detector for determination of acetamiprid and 6-chloronicotinic acid residues in sweet cherry samples. *Pesticides and Phytomedicine*, 27(4), 321-329. doi:10.2298/pif12043211

Lazić, S., Šunjka, D., Grahovac, N., & Guzsány, V. (2013). Determination of acetamiprid in sweet cherry samples. In: *Proceedings of the 19th International Symposium on Analytical and Environmental Problems* (p. 56). Szeged, Hungary: SZAB.

Mandić, A.I., Lazić, S.D., Okresz, S.N., & Gaal, F. (2005). Determination of the Insecticide Imidacloprid in Potato

(*Solanum tuberosum* L.) and Onion (*Allium cepa*) by High-Performance Liquid Chromatography with Diode-Array Detection. *Journal of Analytical Chemistry*, 60(12), 1134-1138. doi:10.1007/s10809-005-0256-x

Ministry of Health and Welfare of Japan. (1997). *Bulletin 179 (September 1)*. Tokyo, Japan: Ministry.

Obana, H., Okihashi, M., Akutsu, K., Kitagawa, Y., & Hori, S. (2002). Determination of acetamiprid, imidacloprid, and nitenpyram residues in vegetables and fruits by high-performance liquid chromatography with diode-array detection. *Journal of Agricultural and Food Chemistry*, 50(16), 4464-4467.

Official website of the European Commission. Retrieved from www.ec.europa.eu/food/animal/liveanimals/bees/neonicotinoids_en.htm

Park, J.Y., Choi, J.H., Kim, B.M., Park, J.H., Cho, S.K., Ghafar, M.W., Shima, J.H. (2011). Determination of acetamiprid residues in zucchini grown under greenhouse conditions: application to behavioral dynamics. *Biomedical Chromatography*, 25(1-2), 136-146. doi:10.1002/bmc.1529

Pravilnik o maksimalno dozvoljenim količinama ostataka sredstava za zaštitu bilja u hrani i hrani za životinje i o hrani i hrani za životinje za koju se utvrđuju maksimalno dozvoljene količine ostataka sredstava za zaštitu bilja. (2010). *Službeni glasnik RS*, 25/2010.

Sasaki, K., Nakamura, Y., Ninomiya, T., Tanaka, T., & Toyoda, M. (1998). Application of the Bulletin Method for Rapid Analysis of Pesticide Residues on the Analysis of 10 Pesticides Notified in 1997. *Journal of Food Hygienic Society of Japan*, 39(6), 448-452. doi:10.3358/shokueishi.39.6_448

Sekulić, J.S., & Jeličić, S.N. (Prir.). (2013). Sredstva za zaštitu bilja u prometu u Srbiji. *Biljni lekar*, 41(1-2), 11-296.

Sur, N., Pal, S., Banerjee, H., Adityachaudhury, N., & Bhattacharyya, A. (2000). Photodegradation of fenarimol. *Pest Management Science*, 56(3), 289-292. doi:10.1002/(sici)1526-4998(200003)56:3<289::aid-ps119>3.0.co;2-f

Tomlin, C.D.S. (2006). *The Pesticide Manual, 14th ed.* Farnham, UK: British Crop Protection Council.

Razgradnja acetamiprida u plodovima trešanja

REZIME

U cilju praćenja razgradnje acetamiprida u plodovima trešanja u periodu od primene preparata do isteka karence, izvršen je tretman preparatom na bazi ove aktivne materije u preporučenoj dozi. Ogled je postavljen u zasadu srednje kasne sorte trešnje na lokalitetu Stepanovićevo u okolini Novog Sada. Plodovi su uzorkovani osam puta – odmah nakon primene preparata, 2, 4, 6, 8, 10, 12 i 14 dana. Ekstrakcija acetamiprida iz trešanja izvedena je QuEChERS metodom. Za određivanje acetamiprida korišćena je tečna hromatografija sa DAD detektorom (Agilent 1100, United States) i Agilent Zorbax Eclipse C18 kolonom (unutrašnji prečnik 50 mm x 4.6 mm, veličina čestica 1.8 µm). Kao mobilna faza upotrebljeni su acetonitril i 1.5% rastvor CH₃COOH (30/70), sa protokom 1 ml/min, temperaturom kolone 25 °C i injektovanom zapreminom 2,5 µl, dok je kao odgovarajuća talasna dužina usvojena vrednost od 254 nm. Validacija metode je u potpunosti sprovedena u skladu sa zahtevima standarda SANCO/12495/2011 (EU Commission Health and Consumer Protection Directorate-General, 2011). Prosečna vrednost prinosa ekstrakcije acetamiprida iz trešanja proverena na tri nivoa obogaćenja (0.1-0.3 mg/kg) iznosila je 85.4%. Preciznost merenja razmotrena proverom ponovljivosti određivanja acetamiprida izražena je relativnom standardnom devijacijom (RSD) sa vrednošću manjom od 1.61%. U opsegu masenih koncentracija acetamiprida od 0,05 do 2,5 µg/ml postignuta je dobra linearnost odziva detektora sa koeficijentom varijacije od 0,995%. Limit detekcije i kvantifikacije za određivanje acetamiprida u trešnjama prikazanom metodom iznose 5 µg/kg i 14 µg/kg. Tokom ispitivanog perioda koncentracija acetamiprida u trešnjama se smanjivala od 0,592 mg/kg neposredno nakon primene insekticida do 0,111 mg/kg po isteku karence od 14 dana. Analizom je utvrđeno da je sadržaj acetamiprida u uzorcima plodova trešnje nakon isteka perioda karence ispod maksimalno dozvoljene količine za ovu aktivnu materiju propisane Pravilnikom Republike Srbije (0,2 mg/kg) i Evropske Unije (1,5 mg/kg).

Ključne reči: acetamid; trešnje; razgradnja; ostaci