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Доклады 7-ого Конгресса по защите растений

Plant Protection Society of Serbia
Общество по защите растений Сербии

International Organization for Biological Control
-East Palearctic Regional Section (IOBC-EPRS)
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Международная организация по биологической борьбе
- Восточно палеарктическая региональная секция (МОББ-ВПРС)
- Западно палеарктическая региональная секция (МОББ-ЗПРС)

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DETERMINATION OF ACETAMIPRID RESIDUES IN SELECTED VEGETABLE AND FRUIT

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ABSTRACT

Increased use of pesticides has resulted in contamination of the environment causing also many associated long-term effects on human health. Therefore, validated analytical methods that produce reliable results for the assessment of pesticide residues in fruits and vegetables are highly needed. The main objective of this study was validation of the method for the analysis of acetamiprid in tomato and determination of its residues after the application at recommended rates under controlled conditions. Obtained results of acetamiprid half-life in tomato are compared with DT_{50} in sweet cherry. For sample pre-treatment QuEChERS procedure was used. Insecticide determination and quantification were performed by HPLC with diode-array detection (Agilent 1100 Series) and Zorbax Eclipse C18 column (50 mm × 4.6 mm internal diameter, 1.8 µm particle size). This method fulfilled validation criteria described in the European Union guidelines (SANCO 12571/2013), by evaluating the accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ), as well as matrix-effect (ME). The accuracy and precision were satisfactory, showing mean recovery values higher than 80% and precision below 20%, in all cases. The validated method was applied for the analysis of acetamiprid residues in real samples. Half-life of acetamiprid in tomato was 4.33 day and it is quite similar to DT_{50} obtained in the experiment with sweet cherries. On the sixth day after the acetamiprid application residues in tomato were at MRL level, as well as in sweet cherries (according to Serbian MRL, 2010), while the PHI was 14 days.

Key words: acetamiprid, tomato, sweet cherry, residue, DT_{50}

INTRODUCTION

Neonicotinoids are the fastest growing class of insecticides (Muccio *et al.*, 2006). They are systemic insecticides that are quite effective in controlling numerous Hemipteran, Thysanopteran, Coleopteran and Lepidopteran species on a wide range of crops, particularly vegetables and fruits (Kim et al., 2003, cit. Park et al., 2010). After the EFSA scientists have identified a number of risks posed to bees by some of neonicotinoid insecticides, the European Commission has excluded from use plant protection products containing clothianidin, thiamethoxam or imidacloprid in crops attractive to pollinators in next two years emphasizing the awareness of potential harmful impact of the neonicotinoids on honeybees

and their products (EU Commission Regulation No. 485/2013 of 24 May 2013; EFSA, 2013). Furthermore, this regulation does not restrict the application of neonicotinoid insecticide acetamiprid {(E)-N1-[(6-chloro-3-pyridyl) methyl]-N2-cyano-N1-methylacetamidine}.

In the Republic of Serbia, acetamiprid is registered for use in tobacco, pepper, cabbage, peach, nectarine and plum for control of aphids, in potato it is used for control of *Leptinotarsa decemlineata*; in tomato for control of *Trialeurodes vaporariorum*; in peas against pea beetle, in onion against leaf miner, in alfalfa against Lucerne leaf-beetle, in apple against leaf aphids, apple moth and apple leaf miner, in pear for control of pear sucker, in cherry and sweet cherries it is used against *Rhagoletis cerasii*, while in vine grape plantations it is used against grape berry moth and grape moth (Sekulić and Jeličić, 2013). Efficiency of products based on acetamiprid led to its intensive use that increased the risk of its residues occurrence in fruits and vegetables. Most of these fruits and vegetables are to a large extent used in fresh conditions without previous thermal treatment. Pre-harvest interval (PHI) for acetamiprid in these cultures is from 14 to 28 days.

Maximum Residue Limits (MRLs) for pesticide residues in fruits and vegetables in our country are stipulated by the Regulations on the maximum allowable quantities of pesticide residues in food and feed for which the maximum allowable quantities of pesticides residues are determined (Official Gazette, RS No. 29/2014). MRL for acetamiprid in tomato established by this Regulation is 0.2 mg/kg and it is entirely in accordance with EU regulations. In relation to sweet cherries, the previous version of the Official Gazette RS from 2010 prescribed the allowable acetamiprid level in sweet cherries of 0.2 mg/kg, while in the new document from 2014, the level is harmonized with EU pesticides database and it is 1.5 mg/kg.

The mainly employed technique for the extraction of pesticides from fruits and vegetables in recent decades is the QuEChERS (Anastassiades et al., 2003). This method involves liquid partitioning with acetonitrile followed by a dispersive SPE clean-up with primary secondary amine and with or without graphitized carbon black (GCB) (Lehotay et al., 2005).

The main objective of this study was validation of the method for the analysis of acetamiprid in tomato and determination of its residues after application at recommended rate under controlled conditions. Obtained results of acetamiprid half-life in tomato will be compared with DT_{50} in sweet cherry, determined in our previous experiment (Lazić et al., 2014).

MATERIAL AND METHODS

Reagents and materials

Acetonitrile HPLC grade and CH_3COOH were obtained from J.T. Baker, Germany. Pesticide standard of acetamiprid was analytical grade (Dr Ehrenstorfer, Augsburg, Germany). Ultra pure water for HPLC analysis (TKA, Germany) was used. Standard was dissolved in acetonitril to make a stock solution of 100 μg/ml. Stock solution was diluted with acetonitrile to make working standard solutions (0.125-1.5 μg/ml) and stored at 4 °C in the dark. For matrix-matched calibration, standards were prepared in the same concentrations, by adding standard stock solutions in blank tomato matrix extracts. 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).

Field trial and sampling

The trial was set up into greenhouse-grown tomatoes at locality Čelarevo. The product based on acetamiprid, MOSPILAN 20 SP with 200 g/kg acetamiprid active substances was used in order to protect tomato from greenhouse white fly (*Trialeurodes vaporariorum*). The solution was prepared at the recommended concentration, according to the manufacturer's instructions (Sekulić and Jeličić, 2013). The samples (around 1.5 kg) were collected before and immediately after application of acetamiprid, and every second day during two weeks (9 samples).

HPLC analysis

HPLC analysis of acetamiprid residues was carried out with an Agilent 1100 Series system equipped with a diode-array detector (Lazić et al., 2014). HPLC determination was conducted using an Agilent Zorbax Eclipse C18 column (50 mm × 4.6 mm internal diameter, 1.8 μm particle size). Mobile phase was acetonitrile and 1.5% CH_3COOH in ultrapure water (30/70) in an isocratic elution at the flow rate of 1.0 ml/min. The column temperature was maintained at 25 °C. Volume of 2.5 μl was injected with auto sampler. Detector wavelength was 254 nm.

Extraction and clean-up procedure

For the extraction of acetamiprid from tomato QuEChERS method (EN 15662 version 2.2, 2008) was used. At 10 g homogenized sample, 10 ml of acetonitrile

was added and vigorously shaken for 1 min. After that, a mix of buffered salts was added and again shaken for 1 min and centrifuged for 5 min at 3000 rpm. Aliquot of 6 ml of the upper acetonitrile layer was transferred to 15 ml centrifuge tube containing the sorbent, mixture of primary-secondary amine (PSA) and magnesium sulphate. The tube was vigorously shaken for 1 min and then centrifuged at 3000 rpm for 5 min. An aliquot of the final upper layer was evaporated to dryness, dissolved in 1 ml of acetonitrile, filtered through a 0.45 μm membrane filter and transferred into an autosampler vial for analyses.

Validation of the analytical method

The method for quantitative analysis of acetamiprid in tomato was validated in terms of accuracy, precision, linearity, limit of detection (LOD) and limit of quantification (LOQ), as well as matrix-effect (ME), in accordance with Document SANCO/12571/2013.

RESULTS AND DISCUSSION

Neonicotinoid's residues in different matrices are usually determined by liquid chromatography LC with diode array detection (Obana et al., 2002; Lazić et al., 2012, 2013), since direct analysis by gas chromatography is unsuitable due to their low volatility and high polarity. Nowadays, they are determined by LC-MS-MS and LC/TOF-MS in vegetables and fruits (Ferre et al., 2005; Jansson et al., 2004; Park et al., 2011; Lazić et al., 2014a).

The validation of the chromatographic method for determination of acetamiprid was carried out using HPLC/DAD under previously described conditions. The linearity of the calibration curve was examined using five calibration solutions prepared in acetonitrile.

The calibration curve was obtained by plotting peak areas in 'y' axis against concentrations of the pesticide in 'x' axis within the investigated range (Figure 1) of concentrations. Each solution was injected in triplicates. The linearity was good with a high correlation coefficient of R^2=0.999.

The obtained LOD and LOQ were 5 μg/kg and 14 μg/kg, respectively. This method provides detection and quantification limits lower than the MRL established by the EU (Europe Commission, 2010) and the Serbian Regulations for acetamiprid in tomato of 0.2 mg/kg. The precision values for the method, expressed as repeatability (relative standard deviation-RSDr) of peak area (n=6), was less than 1.0%.

For determination of acetamiprid recovery, untreated tomato samples (10 g) were spiked with acetamiprid at three concentration level, from LOQ-0.3 mg/kg. Samples were allowed to stand for 30 min, prior to extraction

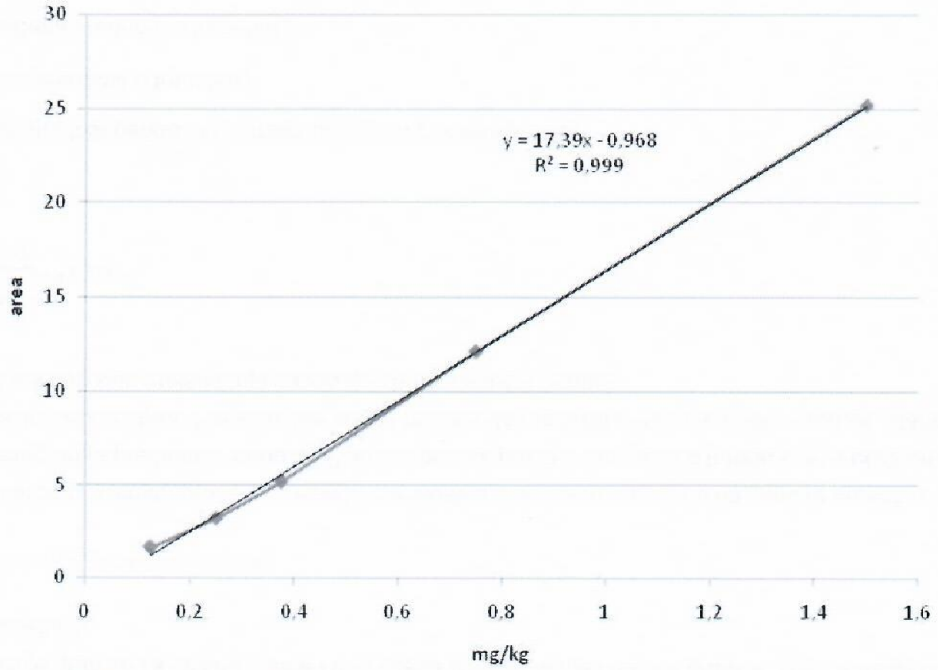

Figure 1. Calibration curve for acetamiprid standards in acetonitrile

by QuEChERS method. Figure 2 presents chromatogram of acetamiprid in tomato matrices.

The mean recovery of acetamiprid in tomato was 96.7±1.1%. According to the EU validation guideline for pesticide residues, mean recovery values should be within the range of 70–120% at each spiking level with acceptable RSDs≤20%. Our previous studies validated the method of the insecticide acetamiprid determination in sweet cherries. The average recovery was 85.4% with RSD=2.5% (Lazić et al., 2014). Matrix effect (suppression or enhancement) was evaluated through the matrix effect percentage (%ME) calculation. This calculation was carried out in accordance with the literature report (Ferrer et al., 2005) as the percentage of the difference between the slopes values of the matrix-match calibration curve and the solvent one. The effect of tomato matrix to acetamiprid signal was 98.04% and there was no observed matrix-effect. In this study, no matrix effect is considered as the values of %ME are into the accepted values (100±20%).

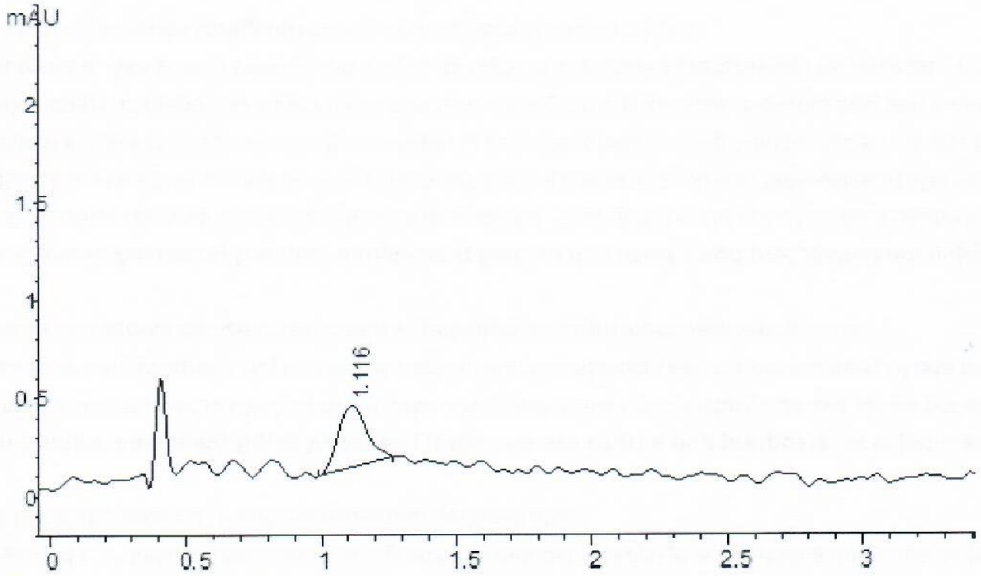

Figure 2. Chromatogram of acetamiprid in tomato sample (0.1 mg/kg)

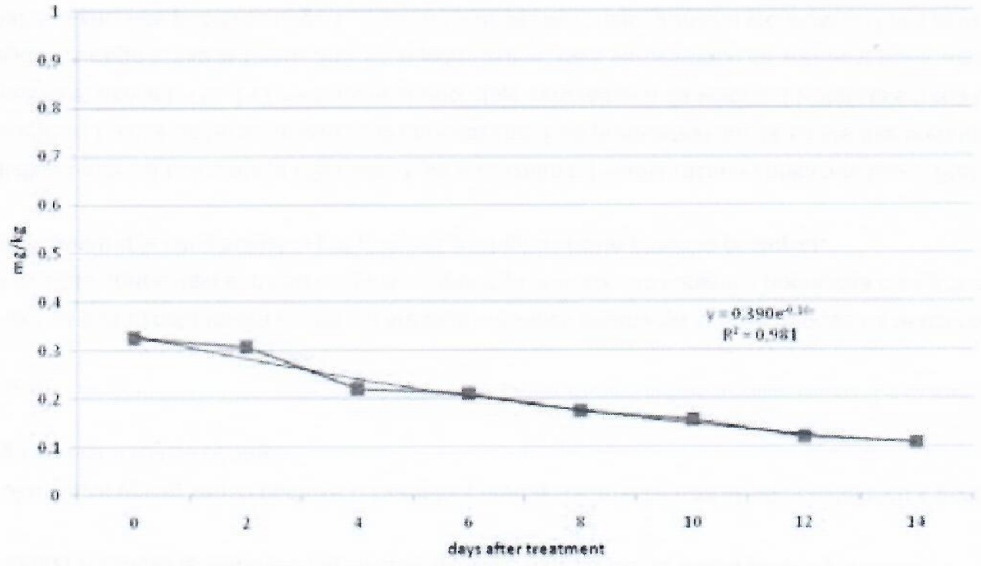

Figure 3. Dissipation of acetamiprid residues in tomato samples during 14 days period

The validated method was applied for the analysis of acetamiprid residues in real tomato samples. Matrix-match calibration was used for the quantification of acetamiprid residues in tomato samples. Pre-harvest interval for acetamiprid in tomato set by Serbian Regulations is 14 days. The maximum residue level of acetamiprid, 0.33 mg/kg, in tomato samples was detected immediately after the application. Two and four days after the application, residues of acetamiprid were 0.31 mg/kg and 0.22 mg/kg, respectively. Six days after the application, acetamiprid content in tomato was at MRL level of 0.2 mg/kg (Figure 3).

In this study half-life (DT_{50}) was calculated from the exponential equation (Figure 3). DT_{50} of acetamiprid in tomato samples obtained in this study was 4.33 days. Half-life for the acetamiprid in medium late variety of sweet cherry, achieved in our previous experiment, was 3.15 days (Lazić et al., 2014).

CONCLUSION

In this study, the method for the determination of acetamiprid residues in tomato using QuEChERS procedure followed by high performance liquid chromatography, was validated. The proposed method proved to be an efficient and sensitive method for the determination of acetamiprid in tomato. Half-life of acetamiprid in tomato was 4.33 day and it is similar to DT_{50} obtained from our experiment in sweet cherries. At 6^{th} day after the acetamiprid application residues were at MRL level. These results are similar to the results from the experiment with sweet cherries. On the sixth day after the acetamiprid application residues in tomato were at MRL level, as well as in sweet cherries (according to MRL from 2010), while the PHI was 14 days.

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