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Original scientific paper

Dissipation and persistence of thiacloprid in pepper fruits

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SUMMARY

This study gives insight into the behavior of the neonicotinoid insecticide thiacloprid in greenhouse production of pepper. Thiacloprid was applied at a concentration of 96 g a.i./ha, as recommended by the manufacturer for aphid control. Degradation of thiacloprid in pepper fruits was evaluated over a ten-day period, starting from the moment of insecticide application. Sample preparation was performed using the QuEChERS method for liquid chromatography with diode array detection for identification and quantification of thiacloprid residues in extracts of pepper samples. The method was validated in accordance with the SANCO/12571/2013 document. The obtained mean recovery value was 83.69%, with RSD 5.05%. Intraday precision was 3.21%. Within a concentration range from 0.01-2.0 μg/ml, thiacloprid showed linear calibration with R^2 0.997%, while the quantification limit of the method was 0.01 mg/kg. The results of a field trial showed that thiacloprid dissipated rapidly from 1.136 mg/kg to 0.321 mg/kg with a loss of 72% in the first two days after application. Throughout the experimental period, thiacloprid residues in pepper fruits basically remained at a stable low level, and no residue exceeding 0.198 mg/kg was detected in the terminal residue experiment, which was below the MRL of 1.0 mg/kg. The half-life (DT_{50}) of thiacloprid in pepper fruits obtained in this study was 4.95 days. Finally, the pre-harvest interval (PHI) for thiacloprid prescribed by Serbian authorities was proved to be safe enough in greenhouse production of pepper.

Keywords: Thiacloprid; Pepper; Dissipation; Residues; Greenhouses

INTRODUCTION

Pepper (*Capsicum annum*) is one of the most important vegetables with great economic significance. It is usually infested by a variety of rapidly reproducing pests, and growers usually apply a combination of several types of pesticides to control them (Lazić et al., 2014a). Intensified production and inadequate application of cultural practices lead to increased insect infestation and diseases, especially under greenhouse conditions of pepper growth. The insecticide thiacloprid is widely used for control of economically highly important pests of pepper, such as aphids. Thiacloprid [(Z)-3-(6-chloro-3-pyridylmethyl)-1,3-thiazolidin-2 ylidenecyanamide] is the chloronicotinyl insecticide that acts selectively on the insect nervous system by inhibiting nicotinic acetylcholine receptors (Li et al., 2016). The structure of thiacloprid is shown in Figure 1.

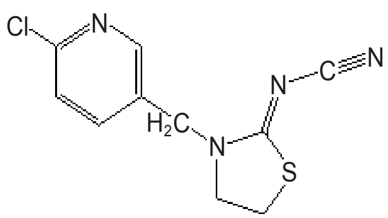

Figure 1. Structural formula of thiacloprid

Thiacloprid is the second member of Bayer's chloronicotinyl insecticide (CNI) family (Jeschke et al., 2001) with a rising usage trend as it has been registered for use in many crops. Previous studies had indicated that thiacloprid could produce delayed lethal and sub-lethal effects on freshwater arthropods at low concentrations (Beketov & Liess, 2008). However, very little information is available on the behavior of thiacloprid in plants and soils (Oliver et al., 2005; Wang et al., 2011).

Due to intensive use of pesticides in vegetable farming, residues may be accumulated at levels higher than those permitted by their respective international maximum residue levels (MRLs). Assessment of dissipation rate of a pesticide after application is a key process for determining the residual behavior of pesticides in agricultural crops and for detecting pre-harvest intervals (PHIs) (Abd-Alrahman & Almaz, 2012). Dissipation rate is one of the most important parameters in assessing the fate of pesticide residues (Li et al., 2016) and it can be used to estimate the time required for bringing residues down below MRLs (Ambrus & Lantos, 2002).

Residue dynamics of thiacloprid have been studied in different matrices (Omirou et al., 2009; Wang et al., 2011; Yu et al., 2007; Jovanov et al., 2013). However, information regarding the behavior of thiacloprid in pepper is still lacking (Li et al., 2016). The MRL for thiacloprid residues in pepper fruits, according to EC (Regulation, 2005) and Serbian legislation (Pravilnik, 2014), is 1.0 mg/kg.

Numerous analytical methods have been described for determination of thiacloprid in different matrices (Ying & Kookana, 2004; Hengel, 2011). Currently, QuEChERS is a method which has been mainly applied for the extraction of different classes of pesticides (Lehotay, 2005; Lazić et al., 2014b). The method has the status of an Official Method of AOAC International (Lehotay, 2007).

In this work, a field study was performed to investigate thiacloprid dissipation in pepper fruits grown in greenhouses. The study presents the results of our examination of a commercial formulation of thiacloprid and its behavior in pepper, and the results of determination of this insecticide's half-life in pepper fruits.

MATERIAL AND METHOD

Reagents and standard solutions

An analytical standard of thiacloprid (purity 99.5%) was obtained from Dr Ehrenstorfer GmbH (Augsburg, Germany). Acetonitrile (HPLC purity) and acetic acid were purchased from J.T. Baker (Darmstadt, Germany), while ultrapure water was obtained from TKA apparatus (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). A stock solution of thiacloprid was prepared in acetonitrile at a concentration of 100 μg/ml. Working standard solutions for HPLC analysis were prepared by further dilution with acetonitrile, achieving concentrations from 0.01 to 2.0 μg/ml. All standard solutions were stored in a refrigerator (4 °C).

Field experiment and sampling

The plants were grown in a greenhouse in 2015. The trial was set up complying with the principles of good agricultural practice, and OEPP/EPPO methods were used for trial design and data processing (EPPO, 2012), as well as the efficacy evaluation of the insecticide in control of aphids in vegetables (EPPO, 2004). The experiments were conducted in three replications. The control plot was allocated some distance from the tested plots and such untreated pepper plants were the source of blank samples in our study of method validation and matrix-matched calibration. The insecticide was applied at the manufacturer's recommended rate of 96 g a.i./ha using a portable hand sprayer. The product was used in the phase of pepper fruits ripening (BBCH 81), at the beginning of aphids' colony formation.

Samples of about 0.5 kg were randomly collected immediately after drying of the spraying mixture and 2, 3, 4, 5, 6, 7 and 10 days after application. Pepper fruits were stored in individual polyethylene bags at -20 °C until extraction (Commission Directive 2002/63/EC).

Sample extraction

The samples were chopped, homogenized and extracted according to the QuEChERS method. In brief, 10.0 g of homogenized pepper sample was weighed into a 50 ml centrifuge tube and 10 ml of acetonitrile was added. The tube was sealed and shaken for 1 min, then votexed for 1 min. To induce the phase of separation and pesticide partitioning, 1000 mg sodium citrate, 500 mg sodium hydrogen citrate sesquihydrate, 4000 mg magnesium sulfate and 1000 mg sodium chloride were added. This was followed by immediate sealing of the tube and its shaking for 1 min, vortexing for 1 min and centrifugation for 5 min at 3000 rpm, and then the tube was sealed, shaken for 1 min, vortexed for 1 min and centrifuged for 5 min at 3000 rpm. For sample clean-up, 6.0 ml of the upper layer was transferred to a tube with primary-secondary amine sorbent (150 mg) and magnesium sulfate (900 mg). After closing the tube, it was vortexed for 1 min and centrifuged for 5 min at 3000 rpm. An aliquot of the upper layer (3 ml) was evaporated to dryness under gentle nitrogen stream, dissolved in 1 ml of acetonitrile, filtered through a 0.45 µm membrane filter and transferred into an autosampler vial (Figure 2).

LC analysis

Liquid chromatographic analysis was performed with an Agilent Technologies Liquid Chromatograph 1100 Series. Chromatographic data were collected and recorded using the Chemstation system software. Separation was carried out using a C_{18} column (50 mm × 4.6 mm internal diameter, 1.8 µm particle size, Agilent Technologies) and isocratic elution of acetonitrile and water acidified with 1.5% CH_3COOH (30/70). The flow rate of the mobile phase was 1 ml/min and column temperature was ambient. The DAD was set at 225 nm with a bandwidth of 4 nm. Each analytical sample was considered in triplicates.

Figure 2. Sample extraction and clean-up procedure

RESULTS AND DISCUSSION

Method validation

The linearity of the method was evaluated by spiking untreated pepper samples with thiacloprid analytical standard because more reliable calibration may be obtained with matrix-matched calibration (European Commission Health & Consumer Protection Directorate-General, 2013). The calibration curve of thiacloprid in pepper matrix in 0.01-2.0 μg/ml concentration range showed an excellent linearity and strong correlation between concentrations and area in the studied range (r^2=0.997).

Blank analyses were also used for interference check from the matrix. Figure 3 shows the chromatograms of blank and matrix-match thiacloprid standard. Absence of any signal at the retention time of thiacloprid (4.601 min) indicated that no matrix compounds were present, which may give a false positive signal.

Recovery assays were performed at three concentration levels: 0.01, 1.0 and 2.0 mg/kg. The samples were processed using the described procedure. At each fortification level, three replicates were analyzed. Quantification of recovery was carried out with the standard dissolved in pepper matrix. The chromatogram of spiked pepper sample

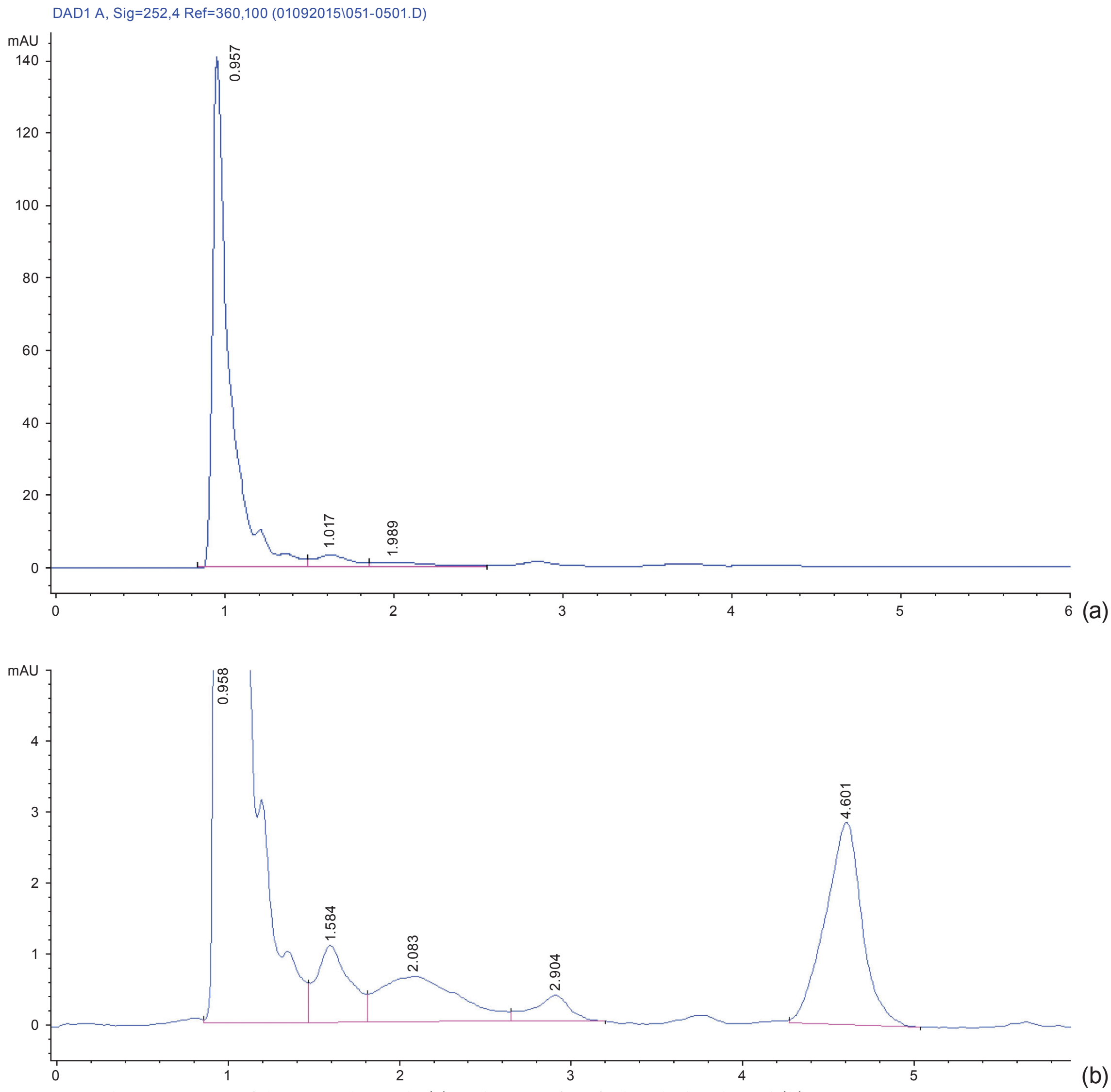

Figure 3. Chromatogram of the control sample (a) and matrix fortified with thiacloprid (b)

(1.0 mg/kg) is shown in Figure 4. In all cases, the recovery of thiacloprid was >70% (83.73-91.14), the average value being 83.69% and RSD=5.05%. These data are generally considered satisfactory for residue determination. Precision, expressed as RSD, was studied by performing repeatability studies (n=6) with the standard dissolved in pure solvent and pepper matrix. The results indicated satisfactory precision of 1.26% and 3.21%. Instrumental LOQ based on S/N of 10:1 was 0.01 mg/kg.

Results of dissipation experiment

Dissipation results are shown in Table 1. The experiment was conducted indoors, the average value of relative humidity was 39.52% and temperature 24.7 °C. Thiacloprid mean residue levels during the sampling period for each application were derived from three sub-samples. The highest residue levels were found in samples taken at the first sampling time 1 h after pesticide application, and the mean value was 1.136 mg/kg. Thiacloprid residue levels kept decreasing in the following period, finally reaching the level of 0.321 mg/kg with dissipation of 72% only 2 days after application, with almost unchanged values on the 3rd day. Over the further three days, thiacloprid residue level was relatively stable, ranging from 0.276 mg/kg on the 4th day to 0.282 mg/kg on the 6th day. At the end of the pre-harvest interval, dissipation of thiacloprid was 83%, and residue level in pepper fruits was 0.198 mg/kg. During the sampling time, thiacloprid content in pepper fruits was far below the MRL of 0.184 mg/kg. Similar results had been reported by Sharma and Perihar (2013). They detected an intensive thiacloprid dissipation (60.42%) on the 2nd day after application.

Table 1. Residues of thiacloprid in pepper fruits

Interval/days	Thiacloprid mg/kg	Loss %	Persistence %
Initial	1.136	0.00	100.00
2	0.321	71.74	28.26
3	0.314	72.37	27.63
4	0.276	75.70	24.30
5	0.277	75.58	24.42
6	0.282	75.16	24.84
7	0.198	82.58	17.42
10	0.184	83.80	16.20

The relationship between residues and time is described by a first-order model, $C_t = C_0 e^{-kt}$, where C_t (mg/kg) is the residue after time t (d), C_0 (mg/kg) is initial residue, and k is dissipation rate constant (d^{-1}). This model was originally adopted to interpret relationships between residues and time and it is widely used to describe the fate of pesticides in soil and plants (Beulke & Brown, 2001).

Half-life (DT_{50}) is defined as the time required for disappearance of 50% of the pesticide (based on initial residue levels after application). For the first-order model,

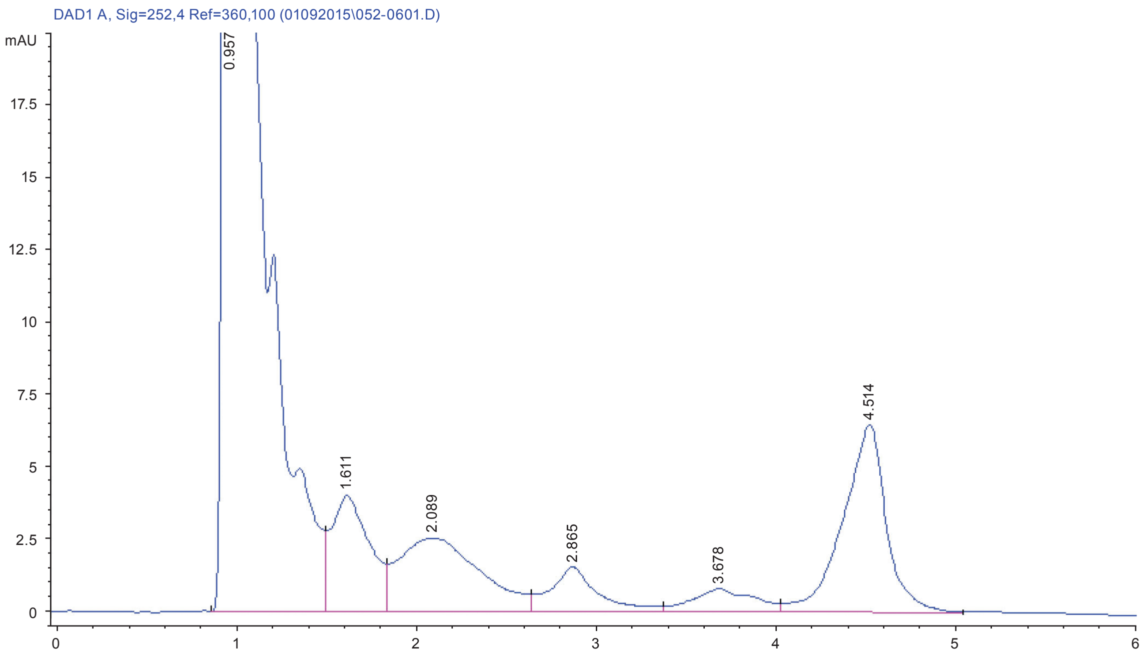

Figure 4. Chromatogram of pepper sample spiked with thiacloprid, 1.0 mg/kg

the DT_{50} was calculated by the following equation: $DT_{50}=\ln2/k$, using dissipation rate constant value.

Dissipation of thiacloprid residues was simulated by a first-order model (Table 2). Degradation monitoring lasted ten days, slightly longer than PHI. Under such conditions, thiacloprid DT_{50} in pepper fruits was 4.95 days.

Table 2. Half-life (DT_{50}) of thiacloprid dissipation

Analyte	Model	Dynamic equation	R^2	DT_{50}
Thiacloprid	First-order model	$y=0.620^{e-0.14x}$	$R^2=0.682$	4.95

In the study conducted by Li et al. (2016) in pepper plants, thiacloprid was applied at 1.5-fold recommended dose of 144 g a.i./ha. The trial was set up in the field. Samples were collected randomly from sampling plots at 0 (2 h after spraying), 1, 3, 7, 10, 14, 21 and 28 days after treatment. The initial residue level of thiacloprid in pepper was 0.503 mg/kg, which is much lower than the established EU MRL. The half-life was 0.81 days.

Considerating the differences in doses of thiacloprid residues and DT_{50} value in our experiment and the trial reported by Li et al. (2016), they can be attributed to different temperature conditions, humidity, and possibly to the insolation level, but also to the plants' habitats. Previous studies had reported that dissipation of pesticide deposits was a complex process depending on various environmental factors, such as temperature, relative humidity and UV irradiation, metabolism and translocation (pesticide penetration and plant growth), application technique and pesticide formulation (Brouwer et al., 1997).

The results of this study indicate that thiacloprid dissipates relatively rapidly in pepper fruits grown in greenhouses as its half-life was 4.95 days. At the end of PHI, thiacloprid residues in pepper plants were far below the MRLs. Finally, the PHI for the thiacloprid insecticide prescribed by the Serbian authorities was proved to be safe enough in greenhouse production of pepper.

REFERENCES

Abd-Alrahman, S.H., & Almaz, M.M. (2012). Dissipation rate of different commercial formulations of propamocarb-hydrochloride applied to potatoes using HPLC-DAD. *Arabian Journal of Chemistry.* Retrieved from , http://www.sciencedirect.com/science?_ob=ArticleListURL&_method=list&_ArticleListID=-923565059&_sort=r&_st=13&view=c&md5=a43fbfbc7a598b18a5968e3026d8cce1&searchtype=a

Ambrus, A., & Lantos, J. (2002). Evaluation of the studies on decline of pesticide residues. *Journal of Agriculture and Food Chemistry,* 50, 4846-4851.

Beketov, M.A., & Liess, M. (2008). Acute and delayed effects of the neonicotinoid insecticide thiacloprid on seven freshwater arthropods. *Environmental Toxicology and Chemistry, 27*(2), 461-470. pmid:18348641

Beulke, S., & Brown, C.D. (2001). Evaluation of methods to derive pesticide degradation parameters for regulatory modeling. *Biology and Fertility of Soils, 33*, 558-564.

Brouwer, D.H., de Haan, M., Leenheers, L.H., de Vreede, S.A., & van Hemmen, J.J. (1997). Half-lives of pesticides on greenhouse crops. *Bulletin of Environmental Contamination and Toxicology, 58*(6), 976-984. pmid:9136663

Commission Directive 2002/63/EC of 11 July 2002 establishing Community methods of sampling for the official control of pesticide residues in and on products of plant and animal origin and repealing Directive 79/700/EEC. L 187/30 (2002). *Official Journal of the European Communities, 16*(7),

EPPO (2004). Aphids on potato, sugar beet, pea, and other vegetables. In *EPPO Standards, Guidelines for the efficacy evaluation of plant protection products, Insecticides & Acaricides,* PP 1/24 (2) (43-46). Paris, France: EPPO.

EPPO (2012). EPPO Standards - Efficacy evaluation of plant protection products - PP1/152(4) - Design and analysis of efficacy evaluation trials. OEPP/*EPPO Bulletin*, *42*(3), 367-381.

European Commission Health & Consumer Protection Directorate-General, (2013). *Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed*. SANCO/12571/2013 Guidelines. Brussels, Belgium: EC.

Hengel, M.J. (2011). Expanded method development for the determination of pesticides in dried hops by liquid chromatography with tandem mass spectrometry. *Journal of the American Society of Brewing Chemists,* 69(3), 121-126. doi:10.1094/ASBCJ-2011-0519-01

Jeschke, P., Moriya, K., Lantzsch, R., Seifert, H., Lindner, W., Jelich, K. .... Etzel, W. (2001). Thiacloprid (Bay YRC 2894): A new member of the chloronicotinyl insecticide (CNI) family. *Pflanzenschutz Nachrichten Bayer, 54*(2), 147-160.

Jovanov, P., Guzsvány, V., Franko, M., Lazić, L., Sakač, M., Šarić, B., & Banjac, V. (2013). Multi-residue method for determination of selected neonicotinoid insecticides in honey using optimized dispersive liquid-liquid microextraction combined with liquid chromatography-tandem mass spectrometry. *Talanta, 111*, 125-133.

Lazić, S., Šunjka, D., Panić, S., Inđić, D., Grahovac, N., Guzsvány, V., & Jovanov, P. (2014b). Dissipation rate of acetamprid in sweet cherries. *Pesticides and Phytomedicine, 29*(1), 75-82.

Lazić, S., Šunjka, D., & Stojanović, I. (2014a). Determination of acetamiprid residues in paprika. In: *Proceedings of II International Congress „Food Technology, Quality and Safety" and XVI International Symposium „Feed Technology"*, Novi Sad, Serbia (pp 521-526), University of Novi Sad, Institute of Food Technology, Novi Sad, Serbia.

Lehotay, S.J. (2005). Quick, easy, cheap, effective, rugged and safe (QuEChERS) approach for determining pesticide residues. In V.J.L. Martinez & F.A. Garrido (Eds.), *Methods in Biotechnology, Vol. 19, Pesticide Protocols* (pp 239-261). Totowa, NJ: Humana Press.

Lehotay, S.J. (2007). Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study. *Journal of AOAC International*, *90*, 485–520.

Li, S., Liu, X., Dong, F., Xu, J., Xu, H., Hu, M., & Zheng, Y. (2016). Chemometric-assisted QuEChERS extraction method for the residual analysis of thiacloprid, spirotetramat and spirotetramat's four metabolites in pepper: Application of their dissipation patterns. *Food Chemistry, 192*, 893-899.

Oliver, D.P., Kookana, R.S., & Quintana, B. (2005). Sorption of pesticides in tropical and temperate soils from Australia and the Philippines. *Journal of Agriculture and Food Chemistry, 53*(16), 6420-6425. **doi:** 10.1021/jf050293l

Omirou, M., Vryzas, Z., Papadopoulou-Mourkidou, E., & Economou, A. (2009). Dissipation rates of iprodione and thiacloprid during tomato production in greenhouse. *Food Chemistry, 116*(2), 499-504.

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 (2014). *Official Gazette RS*,. 29/2014, 37/2014.

Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. (2005). *Official Journal of the European Union, 70*(2), 70.

Sharma, B.N., & Parihar, N.S. (2013). Dissipation and persistence of flubendiamide and thiacloprid in/on tomato and soil. *Bulletin of Environmental Contamination and Toxicology, 90*(2), 252-155. pmid:23229298

Wang, C., Guan, W., & Zhang, H. (2011). Dissipation and residue of thiacloprid in cabbage and soil. *Bulletin of Environmental Contamination and Toxicology, 87*(4), 440-443. pmid:21643830

Ying, G.G., & Kookana, R.S. (2004). Simultaneous determination of imidacloprid, thiacloprid, and thiamethoxam in soil and water by high-performance liquid chromatography with diode-array detection. *Journa of Environmental Science and Health part B, 39*(5-6), 737-746.

Yu, Y.L., Wu, J.I., Stahler, M., & Pestemer, W. (2007). Residual dynamics of thiacloprid in medical herbs marjoram, thyme, and camomile in soil. *Journal of Environmental Sciences, 19*(2), 205-209.

Razgradnja i perzistentnost tiakloprida u plodovima paprike

REZIME

Istraživanje dato u ovom radu sprovedeno je u cilju praćenja dinamike razgradnje insekticida tiakloprida u plodovima paprike iz plasteničke proizvodnje. Tiakloprid je primenjen u količini (96 g a.m./ha) preporučenoj od strane proizvođača za zaštitu paprike od biljnih vaši. Paprika je uzorkovana odmah po sušenju depozita, nakon 2, 3, 4, 5, 6, 7 i 10 dana. Ekstrakcija i prečišćavanje tiakloprida iz plodova paprike izvedena je QuEChERS metodom, dok je za određivanje nivoa ostataka primenjena tečna hromatografija sa DAD detektorom i Agilent Zorbax SB C18 kolona. Validacija metode izvedena je u potpunosti u skladu sa kriterijumima SANCO/12571/2013. Prosečna vrednost prinosa ekstrakcije tiakloprida iz plodova paprike iznosila je 83,69±5,05%, dok je preciznost metode izražena relativnom standardnom devijacijom (RSD) sa vrednošću od 3,21%. Dobra linearnost odziva detektora potvrđena je koeficijentom varijacije od 0,997%.

Limit kvantifikacije za određivanje tiakloprida u paprici ovom metodom iznosi 0,01 mg/kg. Maksimalan nivo ostataka tiakloprida u plodovima paprike gajene u plasteniku utvrđen je po sušenju depozita i iznosio je 1,136 mg/kg. U uzorcima prikupljenim 2. dana od tretiranja, prosečna vrednost ostataka tiakloprida u uzorkovanim plodovima paprike iznosila je 0,321 mg/kg, sa gubitkom od 72%. Pod ovim uslovima proizvodnje, količina tiakloprida se u narednom periodu postepeno smanjivala, sa krajnjom vrednosti od 0,198 mg/kg, znatno ispod MDK od 1,0 mg/kg. Vreme poluraspada (DT_{50}) tiakloprida u plodovima paprike gajene na ovaj način iznosi 4,95 dana. Na osnovu ostvarenih rezultata može se zaključiti da propisana karenca za tiakloprid obezbeđuje bezbednu primenu ovog insekticida u plasteničkoj proizvodnji paprike.

Ključne reči: Tiakloprid; Paprika; Razgradnja; Ostaci; Plastenici